Asian Journal of Agriculture

| Asian J Agric | vol. 7 | no. 1 | June 2022 3
| E-ISSN: 2580-4537 |

Potato diversity photo by Jansky Lab., UW, Madison, WI, USA

Asian Journal of Agriculture

| Asian J Agric | vol. 7 | no. 1 | June 2023 | E-ISSN: 2580-4537 |

Published semiannually **PRINTED IN INDONESIA**

E-ISSN: 2580-4537

9 772580 453132

Asian Journal of Agriculture

| Asian J Agric | vol. 7 | no. 1 | June 2023 |

ONLINE
http://smujo.id/aja

e-ISSN
2580-4537

PUBLISHER
Society for Indonesian Biodiversity

CO-PUBLISHER
Universitas Mulawarman, Samarinda, Indonesia

OFFICE ADDRESS
Department of Agroecotechnology, Faculty of Agriculture, Universitas Mulawarman. Jl. Pasir Balengkong No.1, Kampus Gunung Kelua, Samarinda 75119, East Kalimantan, Indonesia. Tel./Fax.: +62-541-749159/738341, email: editors@smujo.id

PERIOD OF ISSUANCE
June, December

Society for Indonesian Biodiversity

Universitas Mulawarman Samarinda, Indonesia

Published by Smujo International for The Society for Indonesian Biodiversity and Universitas Mulawarman Samarinda, Indonesia

GUIDANCE FOR AUTHORS

Aims and Scope ***Asian Journal of Agriculture*** **(Asian J Agric)** encourages submission of manuscripts dealing with all aspects to optimizing the quality and quantity of both plant and animal yield and final products, including agricultural economics and management, agricultural engineering and mechanization, agronomy and crop science, biotechnology, ecology and ecophysiology, fish breeding, poultry breeding, plants and animals breeding, food science and technology, genetic diversity and breeding, molecular biology, land resources, land use and remote sensing, microbiology, virology and bacteriology, organic agriculture, physiology and nutrition, phytoremediation, plant nutrition, plant pathology and pest management, post-harvest technology, soil sciences, soilless culture, tissue culture technology, and water management.

Article types The journal seeks for: (i) **Research papers**, (ii) **Reviews**, and (iii) **Short communications**. Original full-length research manuscripts are limited to 8,000 words (including tables and figures) or proportional to articles in this publication number (beyond that, it should be with notice). Review articles are also limited to 8,000 words, while Short communications should be less than 2,500 words, except for pre-study (can be more).

Submission The journal only accepts online submissions through the open journal system **(https://smujo.id/aja/about/submissions)** or, for login problems, email the editors at unsjournals@gmail.com (or editors@smujo.id). Submitted manuscripts should be the original works of the author(s). Please ensure that the manuscript is submitted using the template, which can be found at (https://biodiversitas.mipa.uns.ac.id/D/template.doc). The manuscript must be accompanied by a cover letter containing the article title, the first name and last name of all the authors, and a paragraph describing the claimed novelty of the findings versus current knowledge. Please also provide a list of five potential reviewers in your cover letter. They should come from outside your institution and better from three different countries. Submission of a manuscript implies the submitted work has not been published (except as part of a thesis or report, or abstract) and is not being considered for publication elsewhere. When a group writes a manuscript, all authors should read and approve the final version of the submitted manuscript and its revision; and agree on the submission of manuscripts for this journal. All authors should have made substantial contributions to the concept and design of the research, acquisition of the data and its analysis, drafting the manuscript, and correcting the revision. All authors must be responsible for the work's quality, accuracy, and ethics.

Ethics Author(s) must be obedient to the law and/or ethics in treating the object of research and pay attention to the legality of material sources and intellectual property rights.

Copyright If the manuscript is accepted for publication, the author(s) still hold the copyright and retain publishing rights without restrictions. For the new invention, authors must manage its patent before publication.

Open Access The journal is committed to free-open access that does not charge readers or their institutions for access. Readers are entitled to read, download, copy, distribute, print, search, or link to the full texts of articles, as long as not for commercial purposes. The license type is CC-BY-NC-SA.

Acceptance Only articles written in US English are accepted for publication. Manuscripts will be reviewed by editors and invited reviewers (double-blind review) according to their disciplines. Authors will generally be notified of acceptance, rejection, or need for revision within 1 to 2 months of receipt. Manuscripts will be rejected if the content does not align with the journal scope, does not meet the standard quality, is in an inappropriate format, or contains complicated grammar, dishonesty (i.e., plagiarism, duplicate publications, fabrication of data, citations manipulation, etc.), or ignoring correspondence in three months. The primary criteria for publication are scientific quality and significance. **Uncorrected proofs** will be sent to the corresponding author by system or email as .doc or .docx files for checking and correcting typographical errors. The corrected proofs should be returned in 7 days to avoid publication delays. The accepted papers will be published online in chronological order at any time but printed at the end of each month.

Free of charge This publication is dedicated entirely to the advancement of science and technology, therefore author(s), or author institution(s) are not subject to publication fees**. Reprints** The sample journal reprint is only available by special request. Additional copies may be purchased when ordering by email and sending back the uncorrected proofs.

Manuscript preparation Manuscript is typed on A4 (210x297 mm^2) paper size, in a single column, single space, 10-point (10 pt) Times New Roman font. The margin text is 3 cm from the top, 2 cm from the bottom, and 1.8 cm from the left and right. Smaller lettering sizes can be applied in presenting tables and figures (9 pt). Word processing program or additional software can be used; however, it must be PC compatible, use the template, and be Microsoft Word based (.doc or .rtf; not .docx). **Scientific names** of species (incl. subspecies, variety, etc.) should be written in italics, except in italicized sentences. Scientific names (genus, species, author) and cultivar or strain should be mentioned completely for the first time mentioning it in the body text, especially for taxonomic manuscripts. The genus name can be shortened after the first mention, except in early sentences, or where this may generate confusion; name of the author can be eliminated after the first mention. For example, *Rhizopus oryzae* L. UICC 524 can be written hereinafter as *R. oryzae* UICC 524. Using trivial names should be avoided. **Biochemical and chemical nomenclature** should follow the order of the IUPAC-IUB. For DNA sequences, it is better to use Courier New font. Standard chemical abbreviations can be applied for common and clear used, for example, completely written butilic hydroxyl toluene (BHT) to be BHT hereinafter. **Metric measurements** should use IS denominations, and other systems should use equivalent values with the denomination of IS mentioned first. A dot should not follow abbreviations like g, mg, mL, etc. Minus index (m^{-2}, L^{-1}, h^{-1}) suggested being used, except in things like "per-plant" or "per-plot." **Mathematical equations** can be written down in one column with text; in that case, they can be written separately. **Numbers** one to ten are written in words, except if it relates to measurement, while values above them are written in number, except in early sentences. The fraction should be expressed in decimal. In the text, it should be used "%" rather than "percent." Avoid expressing ideas with complicated sentences and verbiage/phrasing, and use efficient and effective sentences.

The title of the article should be written in compact, clear, and informative sentence, preferably not more than 20 words. Name of author(s) should be completely written, especially for the first and the last name. **Name and institution** address should also be completely written with street name and number (location), postal code, telephone number, facsimile number, and email address. We choose local names in Bahasa Indonesia for universities in Indonesia. The mention of "strata" program, should be avoided. Manuscript written by a group, author for correspondence along with address is required (marked with "♥"). **The title page** (first page) should include title of the article, full name(s), institution(s) and address(es) of the author(s); the corresponding authors detailed postage and e-mail addresses (P), and phone (O) and fax numbers (O).

Abstract A concise abstract is required (about 200 words). The abstract should be informative and state briefly the aim of the research, the principal results and major conclusions. An abstract is often presented separately from the article, thus it must be able to stand alone (completely self-explanatory). References should not be cited, but if essential, then cite the author(s) and year(s). Abbreviations should be avoided, but if essential, they must be defined at their first mention. **Keywords** are about five words, covering scientific and local name (if any), research themes, and special methods used; and sorted from A to Z. **Abbreviations** (if any): All important abbreviations must be defined at their first mention there. **Running title** is about five words.

Introduction is about 600 words, covering the aims of the research and provide an adequate background, avoiding a detailed literature survey or a summary of the results. **Materials and Methods** should emphasize on the procedures and data analysis. **Results and Discussion** should be written as a series of connecting sentences, however, for a manuscript with long discussion should be divided into subtitles. Thorough discussion represents the causal effect mainly explains why and how the results of the research were taken place, and do not only re-express the mentioned results in the form of sentences. **Concluding** sentence should be given at the end of the discussion. **Acknowledgements** are expressed in a brief; all sources of institutional, private and corporate financial support for the work must be fully acknowledged, and any potential conflicts of interest are noted.

Figures and Tables of a maximum of three pages should be clearly presented. The title of a picture is written down below the picture, while the title of a table is written above the table. Colored figures can only be accepted if the information in the manuscript can lose without those images; the chart is preferred to use black and white images. The author could consign any picture or photo for the front cover, although it does not print in the manuscript. All images property of others should be mentioned the source. Author is suggested referring to Wikipedia for international boundaries and Google Earth for satellite imagery. If not specifically mentioned, it is assumed to refer to these sources. **There is no appendix**, all data or data analysis is incorporated into Results and Discussions. For broad data, it can be displayed on the website as a supplement.

References Preferably 80% of it comes from scientific journals published in the last 10 years. In the text, give the author names followed by the year of publication and arrange from oldest to newest and from A to Z; in citing an article written by two authors, both of them should be mentioned; however, for three and more authors only the first author is mentioned followed by et al. For example, Saharjo and Nurhayati (2006) or (Boonkerd 2003a, b, c; Sugiyarto 2004; El-Bana and Nijs 2005; Balagadde et al. 2008; Webb et al. 2008). Extent citation should be avoided, as shown with the word "cit." Reference to unpublished data and personal communication should not appear in the list but should be cited in the text only (e.g., Rifai MA 2007, pers. com. (personal communication); Setyawan AD 2007, unpublished data). In the reference list, the references should be listed in alphabetical order. Names of journals should be abbreviated. Always use the standard abbreviation of a journal's name according to the **ISSN List of Title Word Abbreviations** (www.issn.org/2-22661-LTWA-online.php). Please include DOI links for journal papers. The following examples are for guidance.

Journal:

Saharjo BH, Nurhayati AD. 2006. Domination and composition structure change at hemic peat natural regeneration following burning; a case study in Pelalawan, Riau Province. Biodiversitas 7: 154-158. DOI: 10.13057/biodiv/d070213.

The usage of "et al." in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al. 1999. Future of health insurance. N Engl J Med 965: 325-329. DOI: 10.10007/s002149800025.

Book:

Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in the book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds.). Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta*. 50th annual symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.). Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from the internet:

Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4: 187. DOI: 10.1038/msb.2008.24. www.molecularsystembiology.com.

THIS PAGE INTENTIONALLY LEFT BLANK

ASIAN JOURNAL OF AGRICULTURE
Volume 7, Number 1, June 2023
Pages: 1-6

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g070101

Cropping pattern and intensity in the lower belt of Sarpang District, Bhutan

TSHERING TASHI[1,♥], TSHERING TOBGAY[1], TSHERING WANGMO[1], RATU KINLEY[1], SONAM GYELTSHEN[2]

[1]Agriculture Research and Development Center, Samtenling, Department of Agriculture, Ministry of Agriculture and Forests, Royal Government of Bhutan. Sarpang, Bhutan. Tel./fax.: +975-17549901, ♥email: ttashi@moaf.gov.bt
[2]Edith Cowan University. 270 Joondalup Dr, Joondalup WA 6027, Australia

Manuscript received: 17 July 2022. Revision accepted: 13 October 2022.

Abstract. *Tashi T, Tobgay T, Wangmo T, Kinley R, Gyeltshen S. 2023. Cropping pattern and intensity in the lower belt of Sarpang District, Bhutan. Asian J Agric 7: 1-6.* Information on cropping and farming land use is vital for increasing crop production and identifying production gaps, including planning and investment. Increasing cropping intensity through adopting multiple approaches increases crop production rather than pressure on cropland expansion in many countries. In the case of Bhutan, there is a shortage of information on cropping and its intensity in Bhutan. This study presents the status of cropping patterns and cropping intensity at the household level in the Sarpang District using a multistage random sampling technique. Different forms of multiple cropping practices were recorded in the district but were mostly practiced on a small scale and were intermittent. The rice-based cropping pattern was popular in wetlands, while the maize or vegetable-based cropping pattern predominated in dryland. With an average cropping intensity of 112%, the district had a cropping intensity of 97% and 126% for dryland and wetland, respectively. The landholding size revealed a significant inverse relationship with cropping intensity among farmer characteristics. The district's most serious farming problems were a lack of irrigation water and wildlife crop predation. The study recommends a similar study at the national level and developing appropriate intensification of agricultural land use strategy to minimize pressure on cropland expansion in the future. Similarly, agricultural planning and investment merit assessment of crop diversity, crop production resources, cropping intensity gap, and crop mapping.

Keywords: Cropping intensity, cropping pattern, dryland, land use, wetland

INTRODUCTION

Food demand is expected to increase by more than 70% globally by 2050 when the world's population is expected to reach 9 billion people (Godfray et al. 2010; Alexandratos and Bruinsma 2012; Ray et al. 2013). Similarly, Bhutan's population is anticipated to increase by 21.6% in 2047 from 735,553 in 2017 (National Statistics Bureau of Bhutan 2019), so the absolute rise in food demand is rising. The country's most important commodity, cereal, and vegetable self-sufficiency rates were 77.30% and 84%, respectively (Department of Agriculture 2021). The rice self-sufficiency rate was 47% despite being the main food grain. Additionally, the self-sufficiency of essential commodities such as oil, bulb onion, and tomato is 28.8, 18, and 22%, respectively (Department of Agriculture, 2021; Tashi et al. 2022). Almost all agriculture products are worth Nu. 12.7 billion were imported in 2020, accounting for 19% of the total imported value (Department of Revenues and Customs 2020). Given the current status of food supply and consumer demand, it is wise to identify possible solutions for food production growth.

Unlike many other countries, the rugged terrain nature of the Bhutanese landscape favors only about 2.83% of its area suitable for farming, of which lion's share (70.66%) has been brought under cultivation (Ministry of Agriculture and Forests 2019). In addition, the constitution mandates safeguarding a minimum of 60% of the total area under forest cover all the time (Royal Government of Bhutan 2008). The loss of arable land to non-agriculture uses through urbanization and development, and land degradation is another grave concern. That implies the rising food demand will put even more strain on limited arable land, necessitating more food production through intensive farming but through the negotiation of natural resources sustainably. The future will be more challenging as the country's pressure on limited arable land continues to increase due to development, population growth, and natural land degradation. In response to these challenges, solutions such as cropland expansion, yield increase, intensive cropping, and alteration of the natural growing environment must be evaluated and adopted. On the other hand, ensuring minimum adverse environmental and ecological destruction is critical.

Several types of research have demonstrated that increasing cropping intensity could be a viable option for improving food production among numerous strategies (Siebert et al. 2010; Wu et al. 2015; Dias et al. 2016; Meng et al. 2017). That is accomplished by increasing the number of harvests yearly, resulting in higher food supplies without cropland expansion needs. In the next 40 years, Bruinsma (2009) estimated that more than 90% of crop production growth would result from intensification, indicating that cropland expansion may account for only 10% of

production increase. Cropping intensity is a commonly and broadly discussed term that refers to the ratio of the sum of the annual harvested area to total cropland (Bezbaruah and Roy 2002; Siebert et al. 2010; Wu et al. 2015). Wu et al. (2015) suggested that intensive use of existing croplands through increased cropping intensity by increasing the number of cropping cycles or adopting multiple cropping or intercropping can increase food supplies without additional land clearing. This approach may provide an opportunity for Bhutanese farmers to increase the frequency of harvests each year, thereby increasing the food supplies. Unfortunately, agriculture research in Bhutan has mostly focused on increasing yield per unit area of specific crops by screening and adopting high-yielding varieties, applying fertilizer and pesticides, evaluating improved irrigation technologies, and developing value chains. Therefore, increasing food production by cropping intensity has received very little attention in Bhutan. Therefore, it is imperative to explore the potential of cropping intensity in augmenting food production and calibrate its scope in the Bhutanese food production system. Furthermore, while recognizing the limited scope for horizontal expansion, increasing cropping intensity may give a credible strategic opportunity to increase food production.

The present study attempted to assess cropping patterns and determine cropping intensity at the household level in the Sarpang District, as this subject has been less studied in Bhutan. The study area was purposely selected due to the long growing season and similar agroclimatic and geographical conditions representing the large arable land of southern Bhutan. The study reported farming challenges that could be key hurdles to increasing crop production. Understanding cropping intensity at the local level may usher policymakers, researchers, and extension personnel to develop food production strategies and plan initiatives to respond to sustainability challenges and increase food production. In addition, understanding cropping intensity would aid in identifying land-use opportunities in the regions to increase food production.

MATERIALS AND METHODS

Description of the study area

The study was carried out in the Sarpang District, located between 26°42'N and 89°.46'E to 26°47'N and 90°54' E in the subtropical agroecological zone of Bhutan (Figure 1). The district's elevation ranges from less than 200 m to 4,200 m above sea level, where 74% of the district's total area falls below 1,800 m altitude (Department of Forests and Park Services 2016). It experiences hot and humid summer and dry and warm winter, with annual temperatures ranging from 6°C in winter to 35°C in summer. The average annual rainfall in the district is about 5,100 mm (NCHM 2018). Soils are generally sandy loam to loam with a pH of less than 6 with low organic carbon and nitrogen (National Soil Services Center 2001). Approximately 76.5% of total arable land (19,744.54 acres) was cultivated in the district (Ministry of Agriculture and Forests 2020). The estimated population of the district was about 22,000, with 71% living in rural areas (National Statistics Bureau 2017). Crop cultivation and livestock rearing were the predominant economic activities in the district.

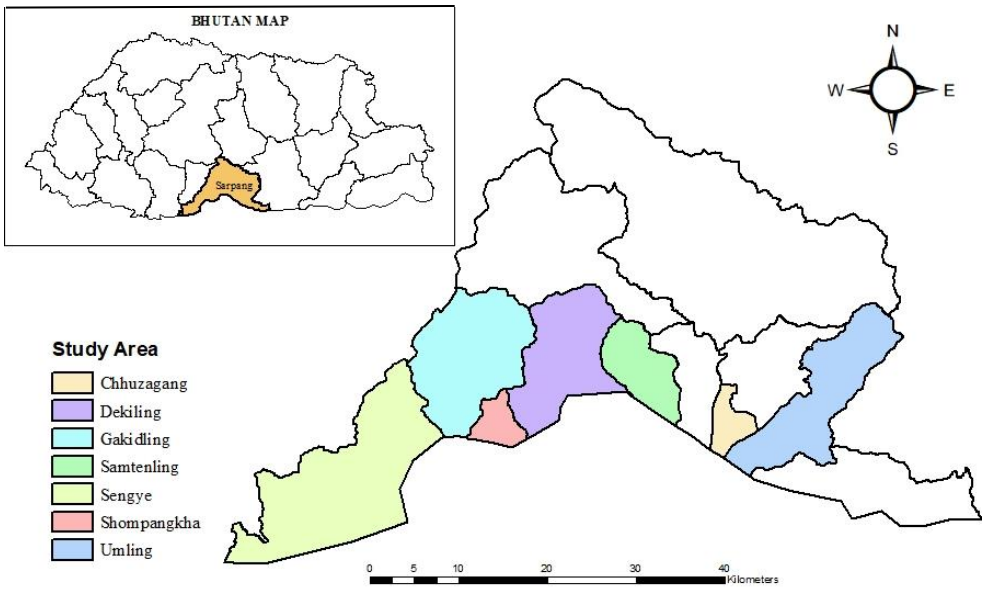


Figure 1. Study area in the Sarpang District, Bhutan

Sampling, data collection, and analysis

The study adopted a multistage sampling technique. The first stage involved sampling six blocks based on altitude (<1,500 m), cultivation area, and farming population. Two villages from each block were randomly chosen in the second stage. In the third stage, samples were drawn at random from all farming households. Next, using pre-tested semi-structured questionnaires, data were collected from 100 households and analyzed using SPSS. Descriptive statistics were mostly used to facilitate the presentation of the findings. Regression analysis examined the relationship between cropping intensity and respondent characteristics. Cropping intensity was calculated as the ratio of total cropped area to net area sown as employed by Siebert et al. (2010), Wu et al. (2015), Atsumi (2016), Gogoi (2016), Rashid et al. (2017), Wu et al. (2018), Waha et al. (2020).

RESULTS AND DISCUSSION

Characteristics of the respondents

More than two-thirds of the respondents (69%) were females. The average age of the respondents was 46 (SD=11.7), representing the age range of 36 to 55. Only 2% of respondents had completed tertiary education, while 76% had no formal education; in rural literacy, 63.6% was reported by the National Statistics Bureau (2017). Most responders (83%) had been farming for more than ten years, indicating a high experience level. There was a significant frequency of relatively small family sizes in the district, with about 65% of respondents having less than six family members and only 4% having more than eight. Annual farming income raged from Nu. 4,000 to 480,000 with an average annual farming income of Nu. 80,660, and 77% of the respondents earn less than Nu. 100,000. Livestock was an integral part of the farming system, as 72% of respondents had livestock components. The average landholding size was 3.26 acres, with 43% owning more than 4 acres and 20% owning less than 2 acres (Table 1). The dryland (*Kamzhing*) size ranges from 0 to 5.5 acres, with an average landholding of 1.75 acres. In comparison, the wetland (*Chuzhing*) size ranges from 0 to 4 acres, with an average landholding size of 1.43 acres (Figure 2).

Cropping information

Almost all subtropical cereals, vegetables, fruits, and other food plants were grown by farmers in the lower belt of Sarpang. Paddy was the most dominant annual crop in the wetland, while vegetables and maize were the most dominant crops in the dryland. Major growing cereals include: paddy, maize millet, mustard, and wheat, while vegetables include: chili, tomato, beans, cole crops, brinjal, pea, cucumber, potato, onion, and leafy vegetables. In addition, garlic, ginger, and turmeric were commonly grown spices, as were fruits such as areca nut, litchi, banana, mango, pineapple, jackfruit, and mandarin. The long growing season allows crops like beans, maize, tomato, and cucumber to be grown twice or thrice in a year as multiple cropping. Although paddy can be grown twice a year as multiple crops, spring paddy cultivation was not very prevalent in farmers' fields.

Table 1. Characteristics of the respondents

Variable		Age	Average
Sex	Male	31	
	Female	69	
Age	25-35	17	45.9
	36-45	34	
	46-55	30	
	56-65	19	
	>65	6	
Education	Tertiary	2	
	Secondary	2	
	Primary	20	
	Uneducated	76	
Farming experience	0-5	4	22.6
	6-10	13	
	>10	83	
Household size	1-5	65	4.5
	6-10	31	
	>10	4	
Income from farming	0-50000	44	80660
	50001-100000	33	
	100001-200000	8	
	200001-400000	11	
	>400000	3	
Livestock	Yes	72	
	No	28	
Landholding	0-0.9	7	3.26
	1-1.9	13	
	2-2.9	24	
	3-3.9	14	
	>4	43	

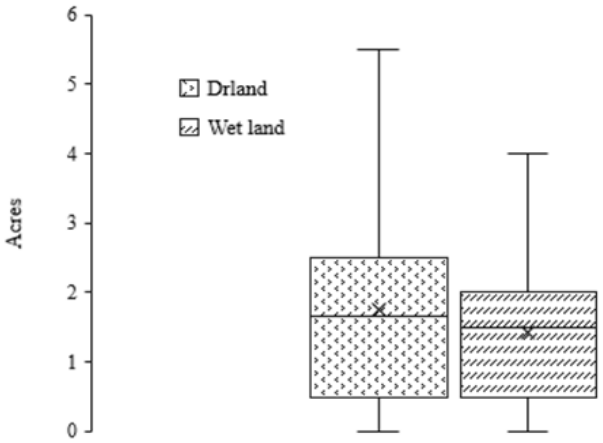


Figure 2. Box plot of landholding

Sequential cropping

Numerous cropping sequences were observed in the studied area; however, only a few significant cropping patterns are reported in Table 2. The most common cropping sequence in dryland was vegetables-maize, practiced by 43% of the respondents. That was followed by maize-chili, vegetables-maize, and vegetables-onion, all used by 39, 23, and 15% of respondents. Maize-gram, vegetables-mustard, and vegetables-fallow were a few of the area's minor cropping sequences. In the maize-vegetables-maize pattern, about 12% of respondents cultivate crops three times a year, and 22% grow crops only once a year. On the other hand, nine paddy-based cropping sequences were recorded in the wetland along with the other two cropping cycles. The paddy occupies the wetland from June-November and is mostly grown as rainfed. After the paddy harvest, various vegetables and other crops were grown until the next paddy season. About 49% of the respondents exclusively use wetlands for paddy cultivation, while 5% do not use wetlands for paddy cultivation. Paddy-vegetables, paddy-chili, and paddy-maize were the most popular wetland cropping sequences, with 34%, 32%, and 25% of respondents, respectively. Minor cropping patterns included maize vegetables and ginger vegetables. Although spring paddy cultivation (February-June) as double cropping was also reported in the district, it was insignificant and limited to governmental initiatives. The district observed appreciable areca nut plantation in wetlands due to irrigation shortage, human-wildlife conflict, and higher economic returns; however, a wetland is exclusively meant for paddy-based cropping farmland in Bhutan (The Land Act of Bhutan, National Land Commission 1 2007).

Multiple cropping

Different forms of multiple cropping practices were common in the Sarpang, but most were sporadic and small-scale (Table 3). Aside from food crops, the plantation of seasonal and annual flowers, shrubs, avenue trees, and other ornamental plants around the residence was popular in the district for aesthetic and religious purposes.

Table 2. Cropping pattern

Dryland		Wetland	
Crops	**Resp. (%)**	**Crops**	**Resp. (%)**
Maize-Vegetables	43	Paddy-Fallow	49
Maize-Chilli	39	Paddy-Vegetables	34
Maize-Maize	23	Paddy-Chilli	32
Vegetables-Onion	15	Paddy-Maize	25
Ginger-Vegetables	13	Paddy-Wheat	12
Potato-Maize	12	Paddy-Mustard	13
Maize-Millet	12	Paddy-Onion	8
Maize-Vegetables-Maize	12	Paddy-potato	6
Maize-Fallow	12	Paddy Lentil	3
Vegetables-Fallow	10	Maize-Vegetables	3
Vegetables-Mustard	9	Ginger-Maize	2
Maize-Gram	3		

Note: Resp.: Respondents

Table 3. Multiple cropping practices in Sarpang, Bhutan

Crops	Type of multiple cropping	Description
Paddy+ Urd beans	Intercropping/Stripe cropping	Common in the district where black gram (urd beans) was planted on terrace bunds of paddy fields as intercrop.
Ginger + Maize	Intercropping	Ginger was planted as the main crop on ridges, while maize was planted in between furrows as an intercrop.
Maize + Potato	Inter/mixed cropping	Sporadic and practiced on a small scale. Mainly potato-based cropping where maize was planted either in furrows or along the border.
Maize+ Bean	Inter/mixed cropping	The beans are sown in the maize field, and the maize plant serves as a stake.
Maize + gram	Inter/mixed cropping	Grams were grown in the maize field as intercrop
Vegetables	Stripe/intercropping	A common practice in the district. For example, beans, chili, cole crops, tomato, leafy vegetables, etc.
Paddy+Lentil	Relay cropping	Lentil was sown before the harvested paddy or after the harvest of the paddy.
Mango, Litchi, Avocado, banana, jackfruit, lime, guava, etc.	Mixed cropping	Mixed fruit planting around the house plot was very common in the district.
Areca nut + beetle leaves	Intercropping	The beetle leave was intercropped with the areca nut plant, which provides support for climbing
Areca nut + banana+ fodder /pineapples	Multi-storied	Very common in areca nut plantations. Fodder/pineapple was grown as a ground crop
Fodder trees	Alley cropping	Random planting of fodder trees such as *Ficus*, *Gmelina*, *Leucaena*, *Bauhinia*, *Morus*, etc., along the periphery of the field

Cropping intensity and land use

Cropping intensity refers to the number of crops grown in the same field over a single agricultural year. Table 4 shows cropping intensity by land type, farm size, and season for 292.85 acres of net cultivated area. Cropping intensity in the district was 112%, which is lower than that of Asia (130%) and the world (126%) (Wu et al. 2018). Wetland cropping intensity was 23% higher than that of dryland. Paddy alone accounts for 75.4% of wetland cropping intensity. Almost half of the farmers left the wetland idle after the rice harvest, implying that most of the wetland remained underutilized for almost half of the year. Thus, taking advantage of this period can boost cropping intensity while supplying temperate regions with winter vegetables. Moreover, oil and legumes were among Bhutan's most commonly imported agricultural commodities, which can be easily integrated into paddy cropping systems (Department of Revenues and Customs 2020). Maize and vegetable-based cropping patterns were found popular in dryland. The average cropping intensity between June and November was 39 and 95%, while November to June was 58 and 31% for dry and wetlands, respectively. The cropping intensity in the dryland from November-June was higher than that of June-November by about 50%. On the contrary, the wetland's November-June cropping intensity was 67% lower than June-November. Therefore, cropping intensity was approximately 50% higher in June-November than in November-June.

It is important to assess the potential cropping intensity based on the availability of resources and local climatic factors and determine the cropping intensity gap. However, it is also important to examine increased cropping intensity's negative environmental and ecological repercussions (Wu et al. 2015). Increasing cropping intensity in the district demands appropriate solutions to major challenges reported by farmers, such as irrigation water shortages, wildlife depredation, high pest and disease incidences, and hot and humid summer, as well as sustainable soil and plant health management. Similarly, improved technology, such as heat and water-logging tolerant crop varieties, pest-disease-resistant crops, short-duration crops, rain shelters, and protected agriculture, are recommended to increase cropping. In addition, socio-economic issues such as the farmers' income, investment, return, profit, and risk-bearing ability must be considered. On the other hand, 4,647.49 acres of uncultivated arable land (23.5%), including 3,351.48 acres of fallow land in the district, could be easily brought under cultivation (Ministry of Agriculture and Forests 2020). Therefore, a comprehensive study on fallow land could provide insights into developing appropriate interventions and using Bhutan's scarce arable land.

Table 4. Cropping intensity in Sarpang, Bhutan

Farm size (acres)	Net cultivated area (Acre)		Gross cropped area (Acre)		Cropping intensity (%)		
	Dryland	Wetland	Dryland	Wetland	Dryland	Wetland	Average
0-1	20.96	11.34	25.70	17.26	123	152	137
>1-2	20.05	68.13	23.47	95.35	117	140	129
>2-3	85.90	30.25	77.70	32.95	90	109	100
>3	44.72	11.50	26.20	11.90	59	103	81
June-Nov	171.63	121.22	66.93	115.16	39	95	67
Nov-Jun	171.63	121.22	99.54	37.58	58	31	45
Overall cropping Intensity					097	126	112

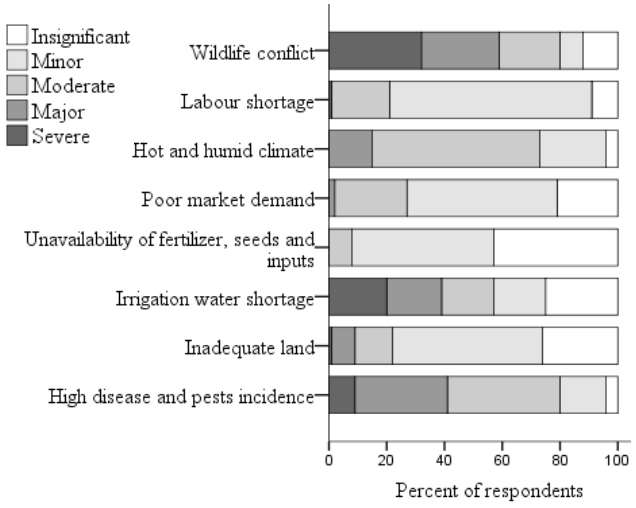


Figure 3. Farming challenges

Cropping intensity and respondents' characteristics

The extent of cropping intensity was studied using the respondents' education, farming experience, income, and landholding size. Multiple regression results demonstrated a significant effect on cropping intensity ($F(4.95) = 8.65$), $p<0.001$), with $R2 = 26.7$, implying that the considered factors predict 26.7% of the variation. Income and farming experience positively impacted crop intensity, but education and landholding size had a negative impact. Except for landholding size, none of the variables significantly affected cropping intensity. However, the landholding size has a negative effect at a 1% significant level, demonstrating that cropping intensity decreases significantly as landholding increases.

Farming challenges

The study examined farming challenges to better understand farming in the district and identify challenges to further crop intensification and agricultural development. Furthermore, using the Likert scale, the scoring system was employed to evaluate the challenges in farming that directly impact crop selection and the degree of cropping intensity. Human-wildlife conflict and irrigation water shortages were reported as severe by 32% and 20% of respondents, respectively, and as a major challenge by 27% and 19%. About 70% of respondents rated high insect and disease prevalence as a serious to moderate farming issue. At the same time, land, labor, seeds, fertilizers, and poor market demand, were mentioned as minor farming problems. Similarly, nearly half of the respondents rated hot and humid weather conditions with high rainfall during the monsoon in the district as a moderate challenge (Figure 3).

This study is the first of its kind in the district to study cropping and its intensity. It is critical to clearly understand crop diversity, cropping patterns, and intensity while making food production and land use management decisions. The study recorded a range of cropping patterns, with maize-vegetables predominating in dryland and paddy-fallow and paddy-vegetables dominating wetland. The cropping pattern selection techniques were not based on scientific understanding; rather than crop choices were made according to their consumption needs. Thus, awareness and demonstrations on developing scientific cropping patterns are crucial to transform subsistence farming into commercial agriculture. It is evident from cropping intensity (1.12) that, on average, farmland was cultivated only once a year, which indicates ample scope to increase cropping intensity and maximize land use return. Notwithstanding increasing cropping intensity, the resurrection of fallow and uncultivated land is suggested through policies and technology support to realize a secure food and nutrition nation.

ACKNOWLEDGEMENTS

The authors would like to thank Agriculture Research and Development Center, Samtenling, Bhutan, for the support rendered during this research.

REFERENCES

Alexandratos N, Bruinsma J. 2012. World Agriculture Towards 2030/2050. ESA Working Paper (Vol. 12, Issue 12). DOI: 10.22004/ag.econ.288998.

Atsumi KT. 2016. Cropping intensity and seasonality parameters across Asia extracted by multi- temporal SPOT vegetation data. J Agric Meteorol 72: 142-150. DOI: 10.2480/agrmet.D-16-00003.

Bezbaruah MP, Roy N. 2002. Factors affecting cropping intensity and use of fertilisers and high-yielding variety seeds in Barak Valley. Indian J Agric Econ 57 (2): 169-179.

Bruinsma J. 2009. The Resoruce Outlook to 2050. Expert Meeting on How to Feed the World in 2050, June, 1075-1095. https://www.fao.org/publications/card/en/c/f70d2098-c9ad-5b05-8972-cf987e74d681/

Department of Agriculture. 2021. Self-Sufficinecy ad Dietary Energy Supplyof Food Crops in Bhutan (A Status Report) (Issue October). https://www.doa.gov.bt/self-sufficiency-and-dietary-energy-supply-of-food-crops-in-bhutan-a-status-report/

Department of Forests and Park Services. 2016. Land Use and Land Cover of Bhutan. Forest Resources Management Division, Department of Forests and Park Services, MoAF, RGoB. http://www.dofps.gov.bt/wp-content/uploads/2018/07/LULC2016_Maps-and-Statistics.pdf

Department of Revenues and Customs. 2020. Bhutan Trade Statistics 2020. https://www.mof.gov.bt/wpcontent/uploads/2021/05/BhutanTradeStatistic2020_Publication.pdf

Dias LCP, Pimenta FM, Santos B, Costa MH, Ladle RJ. 2016. Patterns of land use, extensification and intensification of Brazilian agriculture Patterns of land use, extensification and intensification of Brazilian agriculture. Glob Change Biol 22 (8): 2887-2903. DOI: 10.1111/gcb.13314.

Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C. 2010. Food security: The challenge of the present. Science 327: 73-77. DOI: 10.1016/j.geoforum.2018.02.030.

Gogoi M. 2016. Cropping Pattern in Sivasagar District, Assam, India: A case study. Intl J Innov Res Dev 5 (10): 278-286.

Meng Q, Wang H, Yan P, Pan J, Lu D, Cui Z. 2017. Designing a new cropping system for high productivity and sustainable water usage under climate change. Sci Rep 7: 41587. DOI: 10.1038/srep41587.

Ministry of Agriculture and Forests. 2019. RNR census of Bhutan. www.doa.gov.bt/wp-content/uploads/2021/03/RNR-Census_2019.pdf

Ministry of Agriculture and Forests. 2020. Agricultural land use statistics for effective decision making (Issue 02). www.moaf.gov.bt/rnr-statistics-on-agricultural-land-use-statistics-for-effective-decision-making

National Center for Hydrology & Meteorology (NCHM). 2018. Royal Government of Bhutan Climate Data Book of Bhutan (Issue May). www.nchm.gov.bt/attachment/ckfinder/userfiles/files/Climate Data Book of Bhutan%2C 2018.pdf

National Soil Services Center. 2001. Technical Report on the DetailedSoil Survey of Bhur Farm, Gelephu; Sarpang (Issue October). https://www.nssc.gov.bt/download/technical-report-on-the-detailed-soil-survey-of-bhur-farm-gelephu-sarpang-2001/

National Statistics Bureau of Bhutan. 2019. Population Projections Bhutan 2017-2047. In National Statistics Bureau (Vol. 296, Issue 7672). https://www.nsb.gov.bt/?dlm_download_category=population-projection-2017-47-national-report

National Statistics Bureau. 2017. Population and Housing Census of Bhutan. In National Report. https://www.nsb.gov.bt/?dlm_download_category=population-and-housing-and-census-2017-national-report

Rashid MHA, Islam ABMJ, Shirazy BJ, Shahidullah SM. 2017. Cropping systems and land use pattern in Rajshahi Region. Bangladesh Rice J 21 (2): 237-254. DOI: 10.3329/brj.v21i2.38209.

Ray DK, Mueller ND, West PC, Foley JA. 2013. Yield trends are insufficient to double global crop production by 2050. PLoS ONE 8 (6): e66428. DOI: 10.1371/journal.pone.0066428.

Royal Government of Bhutan. 2008. The Constitution of The Kingdom of Bhutan. Royal Government of Bhutan. https://www.nab.gov.bt/dz/business/constitution_of_bhutan

Siebert S, Portmann FT, Döll P. 2010. Global patterns of cropland use intensity. Remote Sens 2 (7): 1625-1643. DOI: 10.3390/rs2071625.

Tashi T, Dendup C, Gyeltshen S. 2022. Rice self-sufficiency in Bhutan: An assessment. Asian J Agric Ext Econ Soc 40 (2): 18-28. DOI: 10.9734/ajaees/2022/v40i230842.

The Land Act Of Bhutan, National Land Commission 1. 2007. http://oag.gov.bt/wp-content/uploads/2010/05/Land-Act-of-Bhutan-2007_English.pdf

Waha K, Philipp J, Portmann FT, Siebert S, Thornton PK, Bondeau A, Herrero M. 2020. Multiple cropping systems of the world and the potential for increasing cropping intensity. Glob Environ Change 64: 102131. DOI: 10.1016/j.gloenvcha.2020.102131.

Wu W, You L, Chen KZ. 2015. Cropping Intensity Gaps: The Potential for Expanded Global Harvest Areas. In IFPRI Discussion Paper 01459 (Issue August). http://ebrary.ifpri.org/cdm/ref/collection/p15738coll2/id/129407

Wu W, Yu Q, You L, Chen K, Tang H, Liu J. 2018. Global cropping intensity gaps: Increasing food production without cropland expansion. Land Use Policy 76: 515-525. DOI: 10.1016/j.landusepol.2018.02.032.

ASIAN JOURNAL OF AGRICULTURE
Volume 7, Number 1, June 2023 E-ISSN: 2580-4537
Pages: 7-13 DOI: 10.13057/asianjagric/g070102

Application of hormones and mechanical treatment on breaking the dormancy of G_0 "Granola" (*Solanum tuberosum*) minitubers in Northern Mindanao, Philippines

ROSE LYNN P. MACASAMBAT[1,♥], ROMIL J. TAN[2], RAUL E. MARGATE[2]
[1]Central Mindanao University. University Town Musuan, Maramag, Bukidnon, Philippines. Tel.: +63-883561910,
♥email: roselynnmacasambatpontero@gmail.com
[2]Department of Horticulture, College of Agriculture, Central Mindanao University. Sayre Hwy, Maramag, Bukidnon, Philippines

Manuscript received: 3 August 2022. Revision accepted: 13 October 2022.

Abstract. *Macasambat RLP, Tan RJ, Margate RE. 2023. Application of hormones and mechanical treatment on breaking the dormancy of G_0 "Granola" (*Solanum tuberosum*) minitubers in Northern Mindanao, Philippines. Asian J Agric 7: 7-13.* The study was conducted to identify the best treatment combination for dormancy breaking and sprouting quality for "Granola" minitubers. A comparison was made by wounding and dipping the one-week-old minitubers in different levels of GA_3 and BAP (0, 50, and 100 mg/L). Results showed that either 50 or 100 mg/L of GA_3 solely influenced the average number of sprouts in minitubers while wounding minitubers highly influenced tuber weight loss by increasing the percent loss in minitubers. In combination with factors such as wounding and 50 mg/L of GA_3, it positively influenced the average sprout length, fresh mass of sprouts, and dry mass of sprouts. While the dormancy period and days to dormancy break time (50% minitubers achieved > 2 mm sprout length) were reduced for more than 45 and 50 days, respectively, when factors were combined, such as wounding the minitubers and dipping in the treatment solution having 50 mg/L GA_3 and 50 mg/L BAP. The levels of GA_3 and BAP (50 and 100 mg/L) showed no significant differences in achieving early dormancy breaking and high sprouting quality in G_0 minitubers. Overall, for most of the results in this study, wounding combined with the application of 50 mg/L GA_3 was commendable for dormancy breaking and attaining high sprouting quality of "Granola" minitubers.

Keywords: Dormancy breaking, G_0, Granola, minitubers, *Solanum tuberosum*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the world's most important crops next to wheat and rice in terms of demand and consumption (CIP 2020; Maharijaya et al. 2021). It is grown in almost 155 countries worldwide, and billions of people consume it because of its versatility and nutritional values, such as high in phosphorus and vitamins B1, B2, and C (Gonzales et al. 2016), which are the main reasons why countries consider potato as one of the staple crops. Countries such as China and India are the biggest producer of potatoes worldwide (Statista 2019). On the other hand, in the Philippines, potato production has a fluctuating trend (PSA 2018; 2020). In recent data, from July to September 2020, Cordillera Administrative Region (CAR) was registered as the country's largest potato producer, followed by Davao Region and Northern Mindanao, Philippines (PSA 2020). Even with high production in some regions in the country, it is not enough to supply the increasing demand for potatoes not just in the Philippines but also in other countries since it is inevitable that production may decrease due to techniques that the Philippine potato industry has not yet adapted; specifically, dormancy breaking of tubers.

Potato tubers naturally undergo deep dormancy after reaching physiological maturity (Mani et al. 2017). Depending on the variety, dormancy can last up to two to five months without chemical or physical treatments before sprouting (Wrobel et al. 2017). Countries under temperate climatic conditions often consider long dormancy advantageous. However, shorter dormancy is favored in subtropical countries, like the Philippines, where two planting cycles can be achieved per year (Struik and Lommen 1999). According to Wrobel et al. (2008), shortening or breaking the dormancy is a prerequisite, especially during the seed multiplication program and for rapid postharvest quality testing of tubers. Faster distribution of active tubers all year round is one of the keys to eliminating fluctuating production in the country.

During a visit to Northern Mindanao Agricultural Crops and Livestock Research Complex (NMACLRC) at Dalwangan, Malaybalay, Bukidnon, Philippines, which specializes in producing disease-free tubers, one of their concerns is the long dormancy phase of potato tubers. Remediation for breaking the dormancy, such as subjecting dormant tubers to darkness (Claassens et al. 2005), was examined; however, it caused shorter sprouts; thus, that kind of physical treatment was rejected. With this problem, NMACLRC cannot cater to all the farmers' needs to supply planting materials on the spot. With that, limiting active tuber purchases are made to have enough tubers available to other farmers. This case is difficult since other large potato farms are limited with planting materials; thus, farmers are encouraged to take a portion of their harvest (almost 20-40%) to sustain the next cropping season and induce dormant tubers on their own.

While the effects of phytohormones/chemical treatments such as Gibberellic Acid (GA_3) (Hassani et al. 2014; Mustefa et al. 2017; Deligios et al. 2019), Benzyl-Adenine (BA) (Marjeed and Bano 2006), Ethanol (Wrobel et al. 2017), Rindite with GA_3 (Esztergalyos and Polgar 2020), Cytokinin and GA_3 (Hartmann et al. 2011), and Thiourea (Hosseini et al. 2011) have been studied in breaking dormancy, on the other hand, mechanical/physical treatments have limited attention, such as scarification (slicing/cutting and/or wounding) after harvest. It was also studied by Deligios et al. (2019), which found that cutting tubers also significantly resulted in releasing bud sprouts. However, there are few studies involving tubers' wounding (skin scarring) to break the dormancy (Jansky and Hameruik 2014). Normally, excising of tuber buds and cutting of tubers are involved, but the prior technique (scarring) is out of topic.

Today, no existing protocols alleviate this concern in Northern Mindanao, Philippines. With that, this study used the Granola cultivar since it has a three to four months dormancy phase and is the most produced and purchased at NMACLRC. Furthermore, the study served as a guide in breaking the dormancy of potatoes that can be adapted in Northern Mindanao, specifically in NMACLRC under Potato Laboratory, where the seed multiplication program is prioritized.

MATERIALS AND METHODS

Experimental design

The experiment setup was 2 x 3 x 3 factorial in a Completely Randomized Design. Randomization was done via draw lots during the conduct of the study. Each treatment combination (Table 1) was triplicated with 15 tubers per replication. Experimental treatments were composed of mechanical treatment (wounded and unwounded), GA_3 (0, 50, 100 mg/L) (Esztergalyos and Polgar 2020), and BAP (0, 50, 100 mg/L) (Nuraini et al. 2016).

Treatment application

One-week-old minitubers (15-20 g) were washed using tap water and placed in a tray for 10 minutes to dry excess water before applying the treatments. After that, mechanical scarification was applied by putting the tubers (70 minitubers) in the improvised scarring machine for 80 revolutions/turns. Afterward, wounded and unwounded seed minitubers were placed in plastic meshed bags and immersed in different treatment solutions for one hour (Nasiruddin et al. 2016; Lizarazo-Peña et al. 2020). While minitubers under treatment combination having zero/untreated with GA_3 and BAP (Benzylaminopurine), and wounded and unwounded (T1 and T10) were not soaked in distilled water as it served as the control variable in the experiment (practiced in Northern Mindanao). Minitubers were stored in the seed storage screenhouse of NMACLRC for 100 days. Observations were done twice a week at 3 to 4-day intervals for the whole duration of the study.

Table 1. List of hormone and mechanical treatment combinations

Factors			Treatment combination	Treatment code
Mechanical Scarification (Factor A)	**GA_3 (Factor B)**	**CK (Factor C)**		
A1-Wounded	B1-0 mg/L	C1-0 mg/L	A1B1C1	T_1
		C2-50 mg/L	A1B1C2	T_2
		C3-100 mg/L	A1B1C3	T_3
	B2-50 mg/L	C1-0 mg/L	A1B2C1	T_4
		C2-50 mg/L	A1B2C2	T_5
		C3-100 mg/L	A1B2C3	T_6
	B3-100 mg/L	C1-0 mg/L	A1B3C1	T_7
		C2-50 mg/L	A1B3C2	T_8
		C3-100 mg/L	A1B3C3	T_9
A2-Unwounded	B1-0 mg/L	C1-0 mg/L	A2B1C1	T_{10}
		C2-50 mg/L	A2B1C2	T_{11}
		C3-100 mg/L	A2B1C3	T_{12}
	B2-50 mg/L	C1-0 mg/L	A2B2C1	T_{13}
		C2-50 mg/L	A2B2C2	T_{14}
		C3-100 mg/L	A2B2C3	T_{15}
	B3-100 mg/L	C1-0 mg/L	A2B3C1	T_{16}
		C2-50 mg/L	A2B3C2	T_{17}
		C3-100 mg/L	A2B3C3	T_{18}

Data analysis

Percent sprouting. After 100 days, the sprouted minitubers were recorded, considering that there were 45 minitubers per treatment combination. The data were expressed as a percentage.

Dormancy period. The dormancy period was counted as the number of days from treatment application to sprouting (initiation of bud outgrowth-< 2 mm sprout length) (Hosseini et al. 2011). The average mean time (day) to complete germination/sprouting ($\bar{t}$) formula by Bewley and Black (1943) was used to calculate the mean time/day as of when the dormancy period ended in minitubers per treatment combination.

$$(\bar{t}) = \sum (t \times n) / \sum n$$

Where t is the time in days starting from day 0, the day of sowing (or minituber treatment application), and n is the number of minitubers completing germination/sprouting on day t.

Days to dormancy period break time. Days to dormancy period break time was counted and considered when minitubers in each replication attained at least one sprout with more than 2 mm sprout length (Hosseini et al. 2011). The day has taken to achieve 50% (*E*50) minituber sprouting was calculated using the modified formula of Farooq et al. (2005).

$$E_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{n_j - n_i}$$

Where *N* is the final number of the sprouted minitubers, and n_i and n_j are the cumulative number of sprouted minitubers by adjacent counts at times t_i and t_j, respectively, when $n_i < N/2 < n_j$.

Tuber weight loss. Tuber weight loss was examined using a sensitive digital weighing scale. After treatment exposure, the average weight of one-week-old minitubers was labeled as initial weight, and after termination (approximately 100 days), sprouted tubers were weighed for final weight.

The average number of sprouts per minituber. The average number of sprouts per individual minituber was counted and recorded when 95% (Degebasa 2012) of the minitubers sprouted (100 days after treatment application).

Average sprout length (mm). The initial average sprout length was measured in each tuber after breaking dormancy or having 50% sprouted tubers in each replication (> 7 minitubers). The final average sprout length was recorded after 100 days.

Fresh mass of sprouts (mg/minituber). After 100 days, minitubers were de-sprouted and weighed using a sensitive digital weighing scale for accuracy.

Dry mass of sprouts (mg/minituber). After measuring the fresh weight, the dry weight was recorded after oven drying at 80˚C for three consecutive days. The dry matter percentage was then determined.

RESULTS AND DISCUSSION

Percent sprouting

Table 2 summarizes the Analysis of the Variance of all the parameters in this study. Data showed a high percent sprouting of 97.67-100% regardless of the application of MS, levels of GA_3 and BAP, and interaction among factors. That was proven in the analysis of variance, where it showed that the percent sprouting was not influenced by the treatment combinations applied. However, it was observed that most of the minitubers applied with GA_3 showed a trend of faster sprout emergence. The earliest observation of sprouting was one week after treatment application. This observed trend could be supported by the study of Rehman et al. (2003), who stated that GA_3 "accelerates sprouting emergence and accessory shoots.

Dormancy period

Statistical analysis showed that applying GA_3 into the dipping solution significantly shortens the dormancy period of "Granola" minitubers. Although, when it comes to the concentration of GA_3 (50 and 100 mg/L), there is no significant difference in its level, both have the same effects in the dormancy period attaining an average day of 26.99-27.81 days, much shorter compared to the average dormancy period in control (69.27 days), which shows GA_3 has a sole effect on this parameter compared to the other two individually applied factors. The same results were found by Degebasa (2012), where tubers treated with 40 and 50 ppm shortened the dormancy period by 18 to 20 days on Ethiopian potato varieties.

Table 2. Summary of the ANOVA

SS	Percent sprouting	Dormancy period	Days to dormancy break time	Tuber weight loss (%)	Average number of sprouts	Average sprout length (mm)	Fresh mass of sprouts	Dry mass of sprouts
Factor A	0.78^{ns}	3.54^{ns}	1.10^{ns}	50.58^{**}	0.35^{ns}	90.57^{**}	36.47^{**}	40.09^{**}
Factor B	0.67^{ns}	82.42^{**}	111.61^{**}	3.88^{*}	5.47^{**}	139.31^{**}	69.18^{**}	71.74^{**}
Factor C	2.34^{ns}	1.34^{ns}	1.09^{ns}	0.56^{ns}	1.42^{ns}	2.38^{ns}	2.21^{ns}	1.09^{ns}
A x B	0.13^{ns}	1.68^{ns}	0.31^{ns}	0.11^{ns}	0.40^{ns}	19.28^{**}	6.36^{**}	6.76^{**}
A x C	0.77^{ns}	4.43^{*}	4.30^{*}	0.00^{ns}	0.73^{ns}	0.32^{ns}	0.29^{ns}	0.53^{ns}
B x C	1.10^{ns}	3.94^{**}	2.01^{ns}	1.68^{ns}	1.59^{ns}	1.41^{ns}	1.81^{ns}	1.40^{ns}
A x B x C	1.80^{ns}	3.75^{*}	5.29^{**}	0.08^{ns}	0.58^{ns}	0.40^{ns}	0.89^{ns}	0.84^{ns}
C.V.	2.17 %	18.15 %	15.67 %	26.31 %	28.65 %	21.18 %	31.27 %	30.46 %

When it comes to interaction effects, factors, A (MS) and C (BAP), B (GA_3) and C (BAP), and the combination of these three factors (A x B x C) had a significant influence on shortening the dormancy period. Figure 1 demonstrates the interaction effect of factors A and C. Both levels of MS (wounded and unwounded) and BAP (50 and 100 mg/L) attained a dormancy period ranging from 26.32-27.70 days and almost the same average got by factor B alone.

While on the interaction between two factors B and C (GA_3 and BAP), the mean values under treatments having 50 mg/L and 100 mg/L of both phytohormones attained a dormancy period ranging from 25.71-28.44 days much shorter compared to the treatment where levels of BAP were only applied (Figure 2) (42.15-59.19 days).

On the side of the combination of the three factors, lower mean average day values on dormancy period were achieved when both levels had hormone supplementation regardless if the minitubers were scarified or non-scarified. Figure 3 showed that without the hormone application, Factor A alone did not affect the shortening of the dormancy period in minitubers. Regardless of the levels of hormones (50 and 100 mg/L) in wounded minitubers, the dormancy period was significantly shortened by more than 45 days.

These observations proved that these treatment factors could be used as a remediation for attaining a shorter dormancy period in G_0 "Granola" minitubers. Furthermore, the result in this parameter was in line with the studies of Barani et al. (2009), where the effect of GA_3 was positively correlated to the release of tuber sprouting by hydrolyzing tuber starch into renewable sugars; Aksenova et al. (2012a,b) where CK facilitates tuber dormancy to bud outgrowth; and Ostroshy (2006) which accordingly, wounding minituber skin is effective in the penetration of externally applied to promote hormones.

Days to dormancy break time

Statistical analysis showed factor B (GA_3), the interaction between factors A (MS) and C (BAP), and the combination of these three factors (A x B x C) (Figure 5) attained a positive influence on faster dormancy break time. With GA_3 supplementation alone, it showed that 50 and 100 mg/L have the same effect on dormancy break time. Under this factor, 50% was achieved by 37.41 (50 mg/L) and 34.96 (100 mg/L) days. While in the interaction between factors A and C, dormancy break time was faster when minitubers were scarified regardless of the levels of BAP (34.35-34.54 days). However, the application of BAP alone on unwounded minitubers obtained 35.84-37.35 days, faster than wounded/unwounded minitubers without CK (66.12-68.00 days) (Figure 3).

On the other hand, Figure 4 revealed the significant interaction among the three factors (A x B x C). It was demonstrated that minitubers applied with these treatment combinations are closer to the baseline on average day-35.

This average day (35 days) was used to obtain a clear comparison as to when minitubers under these treatments attained 50% > 2 mm sprouts in just 35 days (50 days or shorter compared to the control in this study-84.69 days). That shows a significant reduction in breaking the dormancy of "Granola" minitubers. In addition, GA_3 was also found to have a strong positive influence on this parameter.

Thus, this finding was about the studies of Hassani et al. (2014), where GA_3 supplementation resulted in "breaking minituber dormancy of potato for a short time"; Suttle (2007), where CK (BA) induced dormancy break and sprouting; and Hosseini et al. (2011) which stated that wounds on minituber skin (thin skin/wounds caused by harvesting) facilitates entry of chemicals, but in this case, hormones.

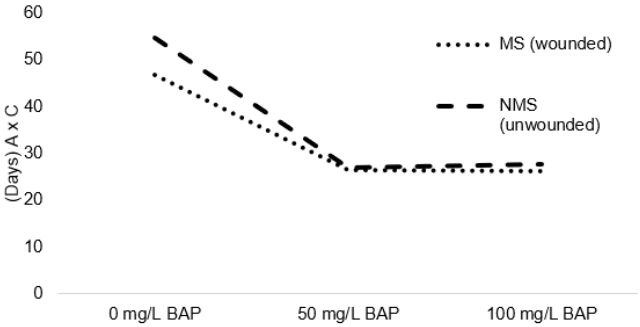


Figure 1. Interaction between Mechanical scarification and BAP levels in breaking the dormancy period (days) of "Granola" minitubers

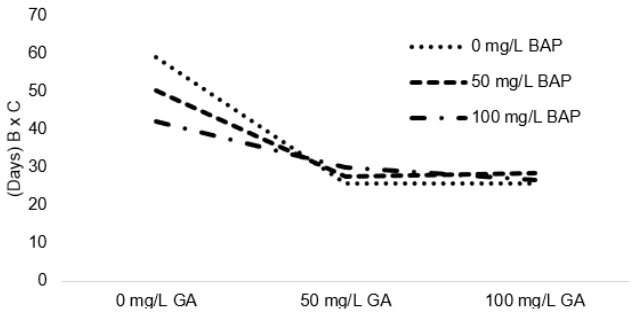


Figure 2. Interaction between GA_3 and BAP in breaking the dormancy period (days) of "Granola" minitubers

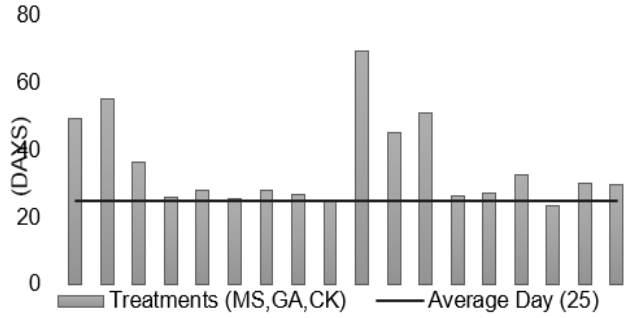


Figure 3. Interaction between three factors-(MS-With Mechanical Scarification; NMS-Without Mechanical Scarification (A); GA (0, 50, 100 mg/L) (B), CK (0, 50, 100 mg/L) (C), in breaking the dormancy period of "Granola" minitubers

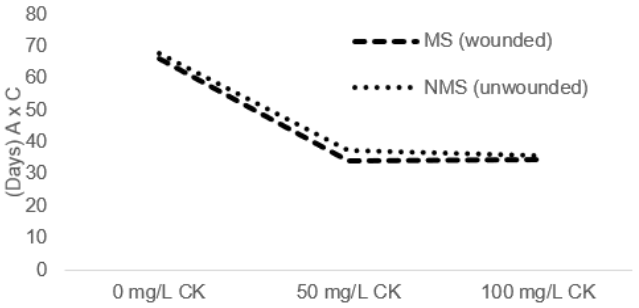


Figure 4. Interaction between Factor A (MS) and Factor C (BAP levels) in days to 50% dormancy break time (> 2 mm sprout length) of "Granola" minitubers

Tuber weight loss

Tuber weight loss was higher when minitubers were subjected to wounding and GA_3. Statistical analysis demonstrated that single factor A (MS) and regardless of factor B (GA3) levels significantly affect the weight loss of minitubers, respectively. In contrast, levels of BAP (Factor C) and interaction among factors did not show a notable difference in this parameter.

Results in this study revealed that wounding applied on minitubers accelerated starch hydrolysis by efficiently delimiting the penetration of GA, which is the main stimulator of accumulating renewable sugars from hydrolyzing the starch content in minitubers (Alexopolous et al. 2008). Furthermore, this result also replicated the findings of Ostroshy (2006), which observed that hormone application is effective on the mechanically damaged skin of potatoes.

With limited attention to previous studies conducted, wounding of minituber skin (MS) has been proven in this result that it has the potential to enhance sprouting, contributing to a higher tuber weight loss. That indicates that when minitubers attain excessive weight loss, they are correlated to vigorous and lengthy sprouts (Ezekiel et al. 2007), which is vital for a positive performance in field propagation.

On the other hand, on Factor B, mean values were found in descending order. In contrast, as the level increased, the mean values were smaller, which is a speculative finding knowing that statistically, this factor is significant. However, compared to another factor (Factor C) and interactions among the factors, the tuber weight loss under the levels of GA_3 (50 and 100 mg/L) was still higher compared to having none (Control-4 %).

Average number of sprouts per minituber

The statistical analysis showed that the number of sprouts was only influenced when GA_3 was supplemented into the dipping solution regardless of the mechanical (Factor A), BAP (Factor C), and the interaction between factors applied. Furthermore, the HSD test revealed that 50 and 100 mg/L of GA_3 were both effective and did not have significant differences in their effect on an average number of sprouts.

This result implies that GA_3 plays an important role in tuber bud outgrowth. Furthermore, high levels of GA_3 (50 mg/L and 100 mg/L) were sufficient in enhancing the number of sprouting in minitubers. These findings showed accuracy to the study of Rehman et al. (2003), where GA_3 supports the acceleration of sprout emergence and is in line with its significant effect on the tuber weight loss parameter where the role of GA_3 in hydrolyzing starch and accumulation of renewable sugars in minitubers (Alexopolous et al. 2008) was mentioned.

In contrast to this result, the study failed to replicate the observation of Suttle (2007), where CK also facilitates sprouting and the effect of mechanical scarification that differed from the results of the earlier parameter (tuber weight loss). Thus, in this case, the addition of GA_3 has been strengthened to be vital in releasing a high sprouting number per minituber compared to the control (no hormone applied).

Average sprout length

Statistical analysis demonstrated that MS (Factor A), specifically wounding, attained a highly significant effect on the sprout length of minitubers. However, without GA_3 (Factor B) supplementation, it was observed that sprouts were shorter even if BAP (Factor C) was applied. With this, based on the analysis, the combination of Factor A (MS) and Factor B (GA3 at 50 mg/L, 100 mg/L) highly influenced the average sprout length of minitubers (Figure 6). While For Factor C, the analysis showed a non-significant effect in the average sprout length.

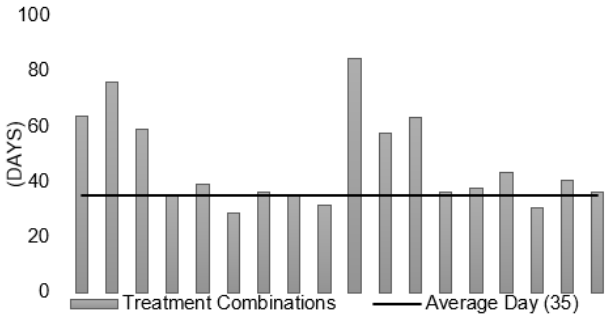


Figure 5. Interaction between three factors-(MS-With Mechanical Scarification; NMS-Without Mechanical Scarification; GA (0,50,100 mg/L), BAP (0,50,100 mg/L), on days to 50 % dormancy break time of "Granola" minituber

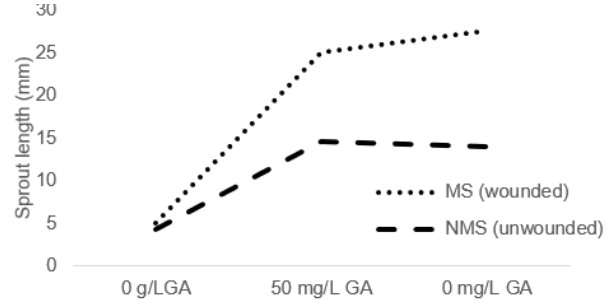


Figure 6. Interaction between Factor A (Mechanical Scarification) and Factor B (GA_3 levels) on average sprout length of "Granola" minitubers

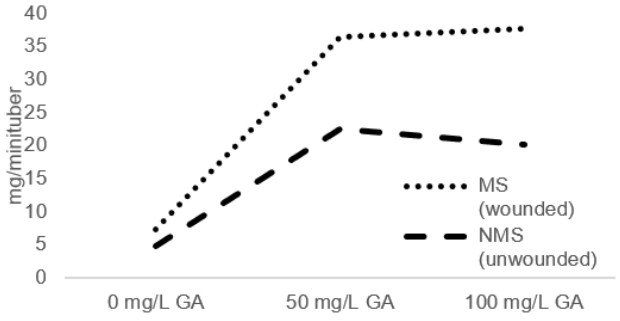


Figure 7. Interaction between Factor A (Mechanical Scarification) and Factor B (50 and 100 mg/L GA_3) on Dry mass of sprout of "Granola" minituber

In this parameter, the observation specifically on the interaction between GA_3 and BAP, where these two phytohormones are known to work together, especially in its application on in vitro seed germination, had created a speculative finding. However, studies involving the incompatibility effect of CK (BAP), specifically on the role of GA_3 in tuber dormancy breaking, are nonexistent today. With this, a thorough study approach on this matter can be done to excavate the factors why CK (BAP) did not complement GA_3 and/or scarred minitubers in this experiment. However, it is still important to note that there are other physiological reasons why CK did not prevail on its positive claims from studies conducted on breaking the tuber dormancy, specifically on tuber sprouting.

In terms of the interaction between Factor A and Factor B (Figure 5), analysis, once again, revealed a highly significant effect in this parameter. Minituber sprout length ranged from 25.14-27.67 mm when minitubers were wounded and dipped at 50 mg/L and 100 mg/L of GA_3. Conversely, minitubers wounded but not dipped in GA_3 got a mean value of 5.04 mm.

This result shows continuality to the findings observed from the tuber weight loss parameter, where it proved that the role of MS greatly maximized the effects of GA_3 by allowing the hormone applied externally to penetrate the internal tissues of minitubers which contributed to a faster and longer sprouting rate (Ostroshy 2006 and Barani et al. 2009).

Fresh mass of sprout

The fresh mass of the sprout (mg/minituber) result was in line with the previous parameter's findings (average sprout length). Statistical analysis revealed that MS (wounding) and GA_3 (50 and 100 mg/L), and in a combination of those two (Factor A x Factor B), highly influenced the fresh mass of sprout of "Granola" minitubers. Treatment means were significantly higher when MS and 100 mg/L of GA_3 was applied regardless of the levels of BAP (Factor C).

This result shows the same trend in its compatibility with MS and GA_3 in allowing the externally applied hormone to maximize its effects on sprouting. With limited attention to studies conducted in this parameter, MS has been proven again to be a reliable treatment for minitubers to have a higher fresh mass of sprouts, indicating higher sprout quality than the control.

Thus, the result in this study shows a relationship to the findings of Ostroshy (2006) and Jansky and Hameruik (2014) and the findings from the previous parameter in this study (average sprout length) where MS and GA_3 are best combined to achieve hormonal effects, tuber viability, and sprout quality.

Dry mass of sprouts

The minitubers attained the highest dry mass means applied with Factor A (wounded) and regardless of the levels of Factor B (GA_3 at 50 mg/L, 100 mg/L).

Statistical analysis demonstrated that MS and GA_3 and the interaction between the two factors (Figure 7) highly influenced the dry mass of sprouts. In comparison, supplementation of BAP on wounded minitubers and/or in a combination of GA_3 did not affect this parameter which is a consistent finding from the previous parameters discussed.

The same trend was shown in the two previous parameters; there is a high mean value on minitubers under the interaction of wounding and levels of GA_3 (either 50 or 100 mg/L) compared to other treatment combinations. That proves the importance of GA_3 as the hormone intervention for sprout quality parameters and the complementary effects of delimiting hormone penetration with MS on minitubers.

REFERENCES

Aksenova NP, Konstantinova TN, Golyanovskaya SA, Sergeeva LI, Romanov GA. 2012a. Hormonal regulation of tuber formation in potato plants. Russ J Plant Physiol 9 (4): 451-466. DOI: 10.1134/S1021443712040024.

Aksenova NP, Sergeeva LV, Konstantinova TN, Golyanovskaya SA, Kolachevskaya OO, Romanov GA. 2012b. Regulation of potato tuber dormancy and sprouting. Russ J Plant Physiol 3: 301-312. DOI: 10.1134/S1021443713030023.

Alexopolous A, Aivalakis G, Akoumianakis KA, Passam HC. 2008. Effect of gibberellic acid on the duration of dormancy of potato tubers produced by plants derived from true potato seed. Postharvest Biol Technol 49 (3): 424-430. DOI: 10.1016/j.postharvbio.2008.02.009.

Barani M, Akbari N, Ahmadi H. 2009. The effect of gibberellic acid (GA_3) on seed size and sprouting of potato tubers (*Solanum tuberosum* L.). Afr J Agric Res 8 (29): 3898-3903.

Bewley JD, Black M. 1943. Seeds: Physiology of Development and Germination. Springer Science+Business Media, New York.

CIP. 2020. International Potato Center. Retrieved 11 April, 2021 from https: //cipotato.org/potato/potato-facts-and-figures/.

Claassens M, Verhees J, Plas L, Ven der Krol A. 2005. Ethanol breaks dormancy of the potato tuber apical bud. J Exp Bot 56 (419): 2515-2525. DOI: 10.1093/jxb/eri245.

Degebasa AC. 2012. Gibberellic Acid for Breaking the Dormancy in Potato Induction. Ethiopian Institute of Agricultural Research, Ethiopian.

Deligios PA, Rapposelli E, Mameli MG, Baghino L, Mallica GM, Ledda L. 2019. Effects of physical, mechanical and hormonal treatments of seed-tubers on bud dormancy and plant productivity. Agron 10 (33): 2-19. DOI: 10.3390/agronomy10010033.

Esztergalyos A, Polgar Z. 2020. The effect of chemical treatments on the tuber dormancy of Hungarian potato cultivars. Potato Res 64: 327-337. DOI: 10.1007/s11540-020-09479-5.

Ezekiel R, Singh B, Kumar D, Mehta, A. 2007. Processing quality of potato varieties grown at two locations and stored at 4, 10, and 12C. Potato J 34 (3-4): 164-173.

Farooq M, Basra S, Saleem B, Nafees M. 2005. Enhancement of tomato seed germination and seedling vigor by osmopriming. Pak J Agric Sci 42: 3-4.

Gonzales IC, Kiswa CG, Bautista AB. 2016. Sustainable potato production in the Philippine Cordillera Region. Intl J Appl Sci 3 (6): 2394-3661.

Hartmann, A, Senning M, Hedden P, Sonnewald U, Sonnewald S. 2011. Reactivation of meristem activity and sprout growth in potato tubers require both Cytokinin and Gibberellin. Plant Physiol 155: 776-796. DOI: 10.1104/pp.110.168252.

Hassani F, Zareian A, Rezvani E. 2014. Effects of chemical treatments on dormancy breaking and some sprouting characteristics of two potato cultivars in different tuber sizes. Euro J Exp Bio 4: 98-102.

Hosseini MB, Afshari RT, Salimi K. 2011. Breaking dormancy of potato minitubers with Thiourea. Potato J 38: 9-12.

Jansky S, Hameruik A. 2014. Rapid cycling of potato tuber generations by overcoming dormancy. Am J Potato Res 92: 148-152. DOI: 10.1007/s12230-014-9415-7.

Lizarazo-Peña PA, Fornaguera-Espinoza F, Ñústez-López CE, Cruz-Gutiérrez NA, Moreno-Fonseca LP. 2020. Effect of Gibberellic Acid-3 and 6-Benzylaminopurine on dormancy and sprouting of potato (*Solanum tuberosum*) tubers cv. Diacol Capiro. Agron Colomb 38 (2): 178-189. DOI: 10.15446/agron.colomb.v38n2.82231.

Maharijaya A, Syukur M, Salma LN, Sanubary UL. 2021. Diversity and performance of eight new promising potato (*Solanum tuberosum* L.) genotypes in Garut District, West Java, Indonesia. Biodiversitas 22: 2848-2858. DOI: 10.13057/biodiv/d220549.

Majeed A, Bano A. 2006. Role of growth promoting substances in breaking potato (*Solanum tuberosum* L.) tuber dormancy. J Agric Soc Sci 2: 175-178.

Mani F, Bettaieb T, Doudech N, Hannachi C. 2017. Physiological mechanisms for potato dormancy release and sprouting: A review. Afr Crop Sci J 22 (2): 155-174.

Mustefa G, Mohammed W, Dechassa N, Gelmesa D. 2017. Effects of different dormancy-breaking and storage methods on seed tuber sprouting and subsequent yield of two potato (*Solanum tuberosum* L.) varieties. Open Agric 2: 220-229. DOI: 10.1515/opag-2017-0023.

Nasiruddin M, Khatun R, Ali FM, Imtiaj A. 2016. Effect of physical and chemical treatments on sprouting of dormant potato tubers. Plant Environ Dev 5 (2): 24-27.

Nuraini A, Sumadiu R, Pratama R. 2016. Application of cytokinins to break the dormancy of G1 potato (*Solanum tuberosum*) seeds. J Kultivasi 15: 202-207. DOI: 10.24198/kultivasi.v15i3.11765. [Indonesian]

Ostroshy M. 2006. Utilization of Tissue Culture Techniques in a Seed Potato Tuber Production Scheme. [Thesis]. Wageningen University, Wageningen. [Netherlands].

PSA. 2018. CountryStat Philippines. Philippine Statistical Authority, Diliman, Quezon City, Philippines. Retrieved 11 April, 2021 from http: //countrystat.psa.gov.ph.

PSA. 2020. CountryStat Philippines. Philippine Statistical Authority, Diliman, Quezon City, Philippines. Retrieved 11 April, 2021 from http: //countrystat.psa.gov.ph.

Rehman K, Lee A, Khabir H, Joung V, Yada R. 2003. Evaluation of Various Chemicals on Dormancy Breaking and Subsequent Effects on growth and yield in potato microtubers under greenhouse conditions. Acta Hortic 619: 375-381. DOI: 10.17660/ActaHortic.2003.619.44.

Statista. 2019. Potato Industry Worldwide-Production. Retrieved 11 April, 2021 from https: //www.statista.com/statistics/382174/global-potato-production/.

Struik PC, Lommen WJM. 1999. Improving the field performance of micro and minitubers. Potato Res 42: 559-568. DOI: 10.1007/BF02358172.

Suttle JC. 2007. Dormancy and Sprouting. In: Vreugdenhil D (Eds.). (Editor)Potato Biology and Biotechnology: Adv IP. 287-309. DOI: 10.1016/B978-044451018-1/50056-7.

Wróbel S, Kęsy J, Treder K. 2017. Effect of growth regulators and ethanol on termination of dormancy in potato tubers. Am J Potato Res 94 (5): 544-555. DOI: 10.1007/s12230-017- 9592-2.

Wróbel S. 2008. Early dormancy breaking of tubers in fast virus diagnostic for new potato cultivars. Prog Plant Prot 48: 552-555.

ASIAN JOURNAL OF AGRICULTURE
Volume 7, Number 1, June 2023
Pages: 14-19

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g070103

Assessment of the acute toxicity of agrochemicals on earthworm (*Aporrectodea caliginosa*) using filter paper contact and soil mixing tests

AHMED FARAHAT EL-ASWAD, MOHAMED RIAD FOUAD♥, MAHER IBRAHIM ALY
Department of Pesticide Chemistry and Technology, Faculty of Agriculture, Alexandria University. Aflaton St., 21545, El-Shatby, Alexandria, Egypt.
Tel.: +20-3-5929846, ♥email: mohammed.riad@alexu.edu.eg

Manuscript received: 1 December 2022. Revision accepted: 30 January 2023.

Abstract. *El-Aswad AF, Fouad MR, Aly MI. 2023. Assessment of the acute toxicity of agrochemicals on earthworm (*Aporrectodea caliginosa*) using filter paper contact and soil mixing tests. Asian J Agric 7: 14-19.* Earthworms are suitable bioindicators of chemical contamination in terrestrial ecosystems. Therefore, adult earthworms (*Aporrectodea caliginosa* Savigny, 1826), which are common in Egyptian rice fields were used to study the side effects of fenitrothion and thiobencarb. Also, it was used two common Egyptian soil types; alluvial soil (clay soil) and the calcareous soil (sandy clay loam soil). Two tests were used; filter paper contact test at 24, 48 and 72 h, and the soil mixing test at 5 and 10 days. The effect of both pesticides on mortality of earthworm was insignificant at 24 h with a low mortality percentage. LC_{50} value of fenitrothion and thiobencarb were 83.16, 288.26, 24.67, and 39.98 µg a.i mL^{-1} at 48 and 72 h, respectively. Based on the LC_{50} values, fenitrothion was 3.5 and 1.6 times more toxic than thiobencarb at 48 and 72 h, respectively. At 5 and 10 days after treatment, respectively, the LC_{50} for fenitrothion in the soil mixing test decreased from 334.27 to 55.45 in clay soil and from (415.90) to (25.00) in sandy soil. Regarding to fenitrothion in soil mixing test, the LC_{50} was reduced from 334.27 to 55.45 in clay soil and from 415.90 to 25.00 in sandy soil at 5 and 10 days after treatment, respectively. Also, the LC_{50} of thiobencarb in clay soil was decreased from 0.93 to 0.41 and from 55.28 to 10.65 at 5 and 10 days, respectively. In general, a positive relation was recorded between the tested pesticide toxicity on earthworm and concentrations, and also exposure time. Fenitrothion was more toxic on earthworm in contact filter paper test than thiobencarb. While, in clay soil, fenitrothion was least toxic to the earthworm in soil mixing test, this could be attributed to the slow degradation in the worms and subsequently less elimination of the metabolites as well as attributed to its higher adsorption and lower desorption in soil.

Keywords: Earthworm, fenitrothion, soil, thiobencarb

INTRODUCTION

Earthworms may be the most significant members of the soil biota because they play major role in the functioning of the soil ecosystem by enhancing soil structure and the decomposition of organic materials. Despite not being numerically dominant in soils, earthworms contribute significantly to overall biomass because of their size. Earthworms play a vital function in structuring and their activities are such that they are incredibly important in sustaining soil fertility in a variety of ways. They create a bigger percentage of biomass (> 80%) of terrestrial invertebrate creatures (Yasmin and D'Souza 2010). The first person to pay attention to the function of earthworms in aerating the soil was Aristotle. In the fauna of soil-dwelling invertebrates, earthworms are more significant organisms (Fouad et al. 2023). Several studies from a long time ago have reported on their favourable effects in the agricultural soils (Badawy et al. 2013; Fouad 2021, et al. 2023).

On the other hand, earthworms are a very significant and helpful species for determining the extent of harmful substance pollution in the soil (Whitacre 2008). As a result, they can serve as appropriate bioindicators of chemical pollution in the soil in terrestrial ecosystems, giving an early warning of a decline in soil quality. It is crucial to do this to safeguard the health of the ecosystem, which is becoming increasingly critical in light of the need to safeguard human health as well as that of other terrestrial vertebrate animals that eat earthworms (Beeby 2001; Yasmin and D'Souza 2010). The primary reason earthworms are used as bioindicators of soil toxicity is that they consume a significant amount of the manure, decomposing litter, and other organic materials deposited in soil, aiding in the conversion of that material into rich topsoil. Additionally, research has revealed that an important pathway for pollution absorption occurs through the epidermis of earthworms (Lord et al. 1980), the earthworms play a crucial part in the bio-magnification process of many soil pollutants since they are a popular prey item for many terrestrial vertebrate species, including birds and small mammals. Therefore, it may be useful to investigate earthworm biomarkers for risk assessment in ecological systems (Yasmin and D'Souza 2010).

Earthworms may be killed, temporarily impaired, or have their behavior and activities inhibited by agrochemicals. Acute, expressed as (LC_{50} or LD_{50}), and chronic toxicities of pesticides on earthworms can be described in laboratory, semi-field, or field circumstances. Additionally, there was no evidence of an influence on the median lethal values, and the time effect (LT_{50}) and concentrations are helpful in determining the negative impact. The calculation of the median lethal time can be used to identify some pesticides with chronic toxicity, such

as poisonous after a prolonged exposure. While polluted soils with higher pollutant concentrations (mortality) can be easily analyzed using the acute test, those with lower pollutant concentrations (sublethal) call for more delicate test methods, such as the reproduction test in the risk assessment (Yasmin and D'Souza 2010).

The harmful affects of various pesticides, including imidacloprid, chlorpyrifos, phoxim, and lambda-cyhalothrin, on earthworms was researched individually and in combination. In the filter paper contact test, imidacloprid, lambda-cyhalothrin, and phoxim were shown to be the most toxic to earthworms, while chlorpyrifos was found to be the least hazardous. The results of the 14-day soil toxicity test revealed that chlorpyrifos and imidacloprid still had the highest levels of toxicity. Lambda-cyhalothrin was found to be comparatively less harmful, while phoxim was shown to be the least poisonous (Cang et al. 2017). Earthworms were exposed to 2,4-D in feed material and in different soil types. The toxic effect of 2,4-D on the earthworm was dose and time dependent. This compound caused 100% mortality of earthworm in soil treated with either 500 to 1000 mg/kg (Singh and Singh 2015). There were no juveniles or cocoons in glyphosate-treated soil. Nevertheless, the soil still contained living earthworms (Correia and Moreira 2010; Singh and Singh 2015).

In this paper, the acute toxicity of the two pesticides (fenitrothion and thiobencarb) commonly used on the rice crop in Egypt on earthworms is studied using the filter paper method and the method of mixing with the soil to reach the best agricultural practices while preserving the environment from pollution.

MATERIALS AND METHODS

Pesticides

Fenitrothion; UPAC name: O, O-dimethyl O-4-nitro-m-tolyl phosphorothioate, Chemical formula: $C_9H_{12}NO_5PS$, Chemical structure: Figure 1, Molar mass: 277.2 g·mol^{-1}, Solubility: Water 0.038 g/L, Product: Technical 97.0% a.i., Production Company: Shandong Chuangying Chemical Co., Available formulations: EC 50%; ULV 100%; WP 25, 40, 50%; G 5%; D 2, 2.5, 3, 5%.

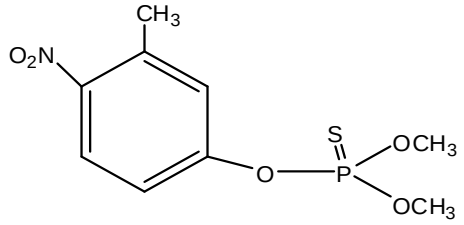
Thiobencarb; UPAC name: S-4-chlorobenzyl diethyl thiocarbamate, Chemical formula: $C_{12}H_{16}ClNOS$, Chemical structure: Figure 1, Molar mass: 257.8 g·mol^{-1}, Solubility: Water 0.030 g/L, Product: Technical 97.0 % a.i., Production Company: Shandong SanYoung Industry Co., Ltd, Available formulations: EC 50%; G 10%.

Soils

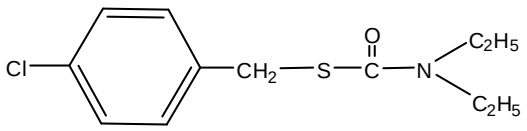
Two types of soil were used, alluvial soil and calcareous soil from Alexandria, Egypt. Soil samples were collected from different places of the surface layer. The physical and chemical properties of the soil were estimated and presented in Table 1 (Badawy et al. 2017; Fouad and El-Aswad 2018; El-Aswad et al. 2019; Aly et al. 2021; Fouad et al. 2021; Fouad 2022, 2023).

Earthworms

The type of earthworm used in this study is one that is found abundantly in rice fields (*Aporrectodea caliginosa* Savigny, 1826). Individual worms were gathered from nearby areas in the Alexandria Governorate and raised in big plastic containers with synthetic soil. Before the studies, the earthworms were kept in the synthetic soil for 15 days (Fouad 2021, et al. 2023).



Fenitrothion



Thiobencarb

Figure 1. Chemical structures of fenitrothion and thiobencarb

Table 1. Physicochemical properties of soils

Chemical properties	Alluvial soil	Calcareous soil
Texture class	Clay	Sandy clay loam
Water holding capacity (mL/100g)	46	38
EC (m mohs/cm) at 25˚C	1.32	5.03
Soil Ph	8.25	8.15
Organic matter content (%)	3.31	1.54
Total carbonate (%)	7.87	44.64
Soluble cations conc. (meq/L)	18.70	50.30
Soluble anions conc. (meq/L)	13.30	50.20

Experiments

Filter paper contact test

Following the contact toxicity protocol, filter papers were treated with pesticide in acetone (0.01, 0.1, 1, 10, 50, 75, 100, and 150 µg/mL) to yield fenitrothion and thiobencarb final concentrations of 7.5, 5, 3.75, 2.5, 0.5, 0.05, and 0.0005 µg/cm^2. After acetone evaporation, four worms were added to each replicate and three replicates to each concentration in addition to the control. The mortality rate was recorded for 24, 48 and 72 hours and the LC_{50} calculated (Fouad 2021; Fouad et al. 2023).

Soil mixing test

In a lab setting, synthetic soil was used to adapt the earthworms. Next, aqueous (35% moisture) solutions of various pesticide formulations were applied to plastic boxes (5 x 10 cm) containing 200 g of the tested soils to produce 200, 100, 10, 1.0, and 0.1 µg/g soil. Then, ten mature earthworms that had been cleaned and aired were added to each box. After 5 and 10 days, the mortality percentage was evaluated. Additionally, using LdP line software, the LC_{50} values for fenitrothion and thiobencarb were determined (Fouad 2021; Fouad 2023).

Statistical evaluation

The Ldp line software ran a statistical analysis on experimental data that was displayed as LC_{50} (Fouad 2021; Fouad et al. 2023).

RESULTS AND DISCUSSION

Testing the toxicity of fenitrothion and thiobencarb on earthworms using filter paper

The findings of an experiment measuring the acute toxicity of fenitrothion and thiobencarb on filter paper showed that the toxicity to earthworms was highly variable (Table 2). The obtained data demonstrated that thiobencarb at a dose of ≤ 1 µg mL^{-1} caused 0% mortality at 48 h and ≤ 50 µg mL^{-1} of both tested pesticides caused 0% mortality at 24 h. The effect of both compounds fenitrothion and thiobencarb on the mortality of earthworm was insignificant with a low mortality percentage at 24 h, suggesting a very weak effect on earthworm. In addition, the concentration of 50 µg mL^{-1} for the tested pesticides caused lower than 50% mortality. The highest tested concentration 150 µg mL^{-1}, gave only 33% and 17% at 24 h for fenitrothion and thiobencarb, respectively. Higher LC_{50} values are less toxic because greater concentrations are required to produce 50% mortality in organisms. Fenitrothion demonstrated the greatest intrinsic toxicity to the worms over at 48-h period, with an LC_{50} value of 83.16 µg a.i mL^{-1} (Table 3). At 72-h interval, the toxicity of thiobencarb increased to the earthworms with an LC_{50} value of 39.98 (22.88-73.61) µg a.i mL^{-1} (0.42 µg cm^{-2}), however, fenitrothion still exhibited the highest toxicity with an LC_{50} value of 24.67 (17.95-33.90) µg a.i mL^{-1} (0.26 µg cm^{-2}). There is a positive association between pesticide toxicity and exposure time, as evidenced by the fact that the toxicity rose as the exposure time grew. Based on the LC_{50} values, the toxicity of fenitrothion at 72 h was 3.4 times more than its toxicity at 48 h and the toxicity of thiobencarb at 72 h was 7.2 times more its toxicity at 48 h. In addition, based on the LC_{50} values, fenitrothion was 3.5 and 1.6 times more toxic than thiobencarb at 48 and 72 h, respectively. From comparing the efficacy of fenitrothion and thiobencarb according to their toxicity indices LC_{50}, it was found that fenitrothion has relative toxicity = 100, with thiobencarb has relative toxicity index of 28.85 and 61.71, compared to fenitrothion at 48 and 72 h, respectively. This is how the relative toxicity was determined;

$$Relative\ toxicity = \frac{LC_{50} for\ the\ most\ effective\ pesticide}{LC_{50} for\ the\ other\ pesticide} \times 100$$

The slope values did not significantly differ between the two tested pesticides. This was due to a similarity in sensitivity of the earthworm individuals to both tested pesticides. The Chi-Square values were 10.35, 65.69 for fenitrothion and 2.76, 17.82 for thiobencarb at 48 h and 72 h, respectively. The p-value ranged from 0.01 to 0.37 for two tested pesticides (Table 3).

It was reported that to calculate LC_{50}, concentration needed to cause 50% mortality after a given period of exposure (Simon-Delso et al. 2018). Therefore, the lethal toxicity and its parameters were calculated only at 48 and 72 h for tested pesticides. However, all of the fenitrothion and thiobencarb concentrations tested caused the earthworm mortality in a concentration-dependent manner. In general, according to system proposed by Roberts and Dorough (1984), which indicated that based on the resulting LC_{50} values of earthworms that exposed to deposits of the chemicals on filter paper for 48 h. It can be stated that fenitrothion would be classified as supertoxic (LC_{50} at 48 h = 0.88 µg cm^{-2}) whereas thiobencarb would be classified as moderately toxic (LC_{50} at 48 h = 303 µg cm^{-2}). In contrast, it has been illustrated that many organophosphates such as fenitrothion, diazinon, azinphos-methyl and malathion are only slightly toxic or may be not toxic to earthworms (Griffiths et al. 1967). Several pesticides, considered only (moderately or relatively nontoxic to mammals), were extremely or very toxic to earthworms; among these compounds were cypermethrin, carbaryl, malathion and benomyl, indicating the unpredictability of pesticide toxicity to different animal species, a fact which complicates the environmental risk assessment to one or more species based on data attained with another (Roberts and Dorough 1984).

Table 2. Toxicity of tested fenitrothion and thiobencarb (Mortality % ±SE) on earthworm by filter paper contact test

Conc. (ppm)	Fenitrothion			Thiobencarb		
	24h	48h	72h	24h	48h	72h
0.01	0.00	0.00	0.00	0.00	0.00	8.33 ±8.33
0.1	0.00	0.00	8.33 ±8.33	0.00	0.00	16.67 ±8.33
1	0.00	0.00	16.67 ±8.33	0.00	0.00	25.00 ±0.00
10	0.00	8.33 ±8.33	25.00 ±14.43	0.00	8.33 ±8.33	33.33 ±8.33
50	0.00	25.00 ±14.43	41.67 ±22.05	0.00	16.67 ±8.33	41.67 ±8.33
75	8.33 ±8.33	41.67 ±8.33	50.00 ±14.43	8.33 ±8.33	25.00 ±14.43	50.00 ±14.43
100	25.00 ±8.33	58.33 ±8.33	83.33 ±8.33	8.33 ±8.33	33.33 ±8.33	58.33 ±22.05
150	33.33 ±8.33	75.00 ±14.43	91.67 ±8.33	16.67 ±8.33	41.67 ±8.33	75.00 ±14.43

Table 3. Tests on evaluated pesticides' toxicity characteristics using contact with filter paper on earthworms

Pesticide	Time (h)	LC_{50} [a] (µg/mL)	Confidence limits at 95%	Slope[b]	χ^2 [c]	P	Relative toxicity[d]
Fenitrothion	48	83.16	71.20-97.16	1.81 ±0.04	10.35	0.306	100
	72	24.67	17.95-33.90	0.75 ±0.04	65.69	0.309	100
Thiobencarb	48	288.26	163.82-512.94	1.04±0.04	2.76	0.367	28.85
	72	39.98	22.88-73.61	0.41 ±0.00	17.82	0.007	61.71

Note: a: Concentration causing 50% mortality. Results of LC_{50} are expressed as mean of three replicates ± standard error (SE). b: Slope of the concentration-mortality regression line ± SE. c: Chi square value. d: Relative toxicity = (LC_{50} level for the most effective pesticide/LC_{50} level for the other pesticide) × 100

Toxicity of tested pesticides on earthworm by soil mixing test

The soil mixing test is more accurate in simulating the natural environment of earthworms. Table 4 shows the mortality percentages of earthworms exposed to fenitrothion (EC 50%) and thiobencarb (EC 50%) at concentrations of 0, 0.1, 1, 10, 100 and 200 µg/g soil at time 5 and 10 days after treatment in clay and sandy clay loam soil. The highest mortality percentage for fenitrothion at 200 µg/g soil, was 68.00 and 88.00 % at 10 days in clay soil and sandy clay loam soil, respectively. When the exposure time was extended, the LC_{50} values for the effects of fenitrothion and thiobencarb on earthworms rose. At 5 and 10 days after treatment, the LC_{50} of fenitrothion was decreased in clay soil from 334.27 to 55.45 and in sandy soil from 415.90 to 25.00. Additionally, at 5 and 10 days, respectively, the LC_{50} of thiobencarb in clay soil reduced from 0.93 to 0.41 and from 55.28 to 10.65. The toxicity of tested pesticides was greater (lower LC_{50}) in clay soil at both time intervals than that in sandy clay loam soil, except fenitrothion at 10 days. Thiobencarb showed the highest toxicity to *A. caliginosa* at 5 and 10 days in clay and sandy soil with the lowest LC_{50} values compared to fenitrothion which exhibited the least toxicities to the worms with the highest LC_{50}values (Table 5). Meanwhile, fenitrothion showed a relatively less toxicity with an LC_{50} value of 55.45 µg/g soil for clay soil and 25.00 µg/g soil for sandy soil. On the other hand, based on the LC_{50} values, the toxicity of thiobencarb in clay soil at 5 days was 360 times and at 10 days was 135 times more than the toxicity of fenitrothion. In addition, thiobencarb in sandy clay loam soil was 7.5 and 2.5 times more toxic than fenitrothion at 5 and 10 days, respectively. From comparing the efficacy of the tested pesticides according to the relative toxicity, thiobencarb has relative toxicity = 100, with fenitrothion has relative toxicity of 0.28 and 0.74 in clay soil and 13.29 and 42.60 in sandy clay loam soil compared to thiobencarb at 5 and 10 days, respectively. It should be noted that fenitrothion was least toxic to the earthworms, this could be attributed to to the slow degradation in the worms and subsequently less elimination of the metabolites. Moreover, the slope value was < 0.8 at all probability levels. The Chi-Square ranged from 6.62 to 32.17 for fenitrothion and from 7.91 to 51.25 for thiobencarb (Table 5).

It was reported that fenitrothion could be slightly toxic or not toxic to earthworms and it was found that thiobencarb has moderate toxicity to earthworms, its LC_{50} is 874 µg/mL (EC 50%) at time 14 day on earthworms. Several herbicides (acetochlor, anilofos, flutamone, pretilachlor, S-metolachlor, and terbutryn) were very toxic in contact toxicity but were low toxic in soil toxicity testing. Therefore, an ecotoxicological assessment of the effects of herbicides on earthworms should be carefully evaluated. Several workers reported that herbicides have adverse effect on the survival of earthworms, as well as its growth and reproduction (Correia and Moreira 2010). Studies conducted by Monsanto researchers reported that no adverse effects were observed when earthworms were exposed to glyphosate residues in soil at rates equal to or greater than labeled rates. Application of simazine and isoproturon have no toxic effect on earthworms (Lydy and Linck 2003; Mosleh et al. 2003). Toxicity of tested pesticides depends on different factors such as soil type (Raj and Syriac 2017), pesticide type (Roberts and Dorough 1984), tested concentrations (Martin 1982), exposure time (Singh and Singh 2015), effects by dermal exposure or ingestion and effects by contact toxicity or soil mixing toxicity (Mosleh et al. 2003; Araneda et al. 2016).

Table 4. Toxicity of fenitrothion and thiobencarb on earthworm (Mortality % ±SE) by soil mixing test

Conc. (ppm)	Clay soil		Sandy clay loam soil	
	5th day	10th day	5th day	10th day
Fenitrothion				
0.1	6.67 ±6.67	20.00 ±11.55	0.00	13.33 ±6.67
1	13.33 ±6.67	26.67 ±6.67	6.67 ±6.67	20.00 ±0.00
10	20.00 ±11.55	33.33 ±13.33	13.33 ±6.67	26.67 ±6.67
100	33.33 ±6.67	46.67 ±17.64	26.67 ±13.33	53.33 ±13.33
200	55.00 ±6.67	68.00 ±6.67	48.00 ±6.67	88.00 ±6.67
Thiobencarb				
0.1	33.33 ±6.67	40.00 ±11.55	6.67 ±6.67	13.33 ±6.67
1	46.67 ±6.67	53.33 ±6.67	13.33 ±6.67	20.00 ±0.00
10	66.67 ±6.67	80.00 ±11.55	20.00 ±0.00	26.67 ±6.67
100	86.67 ±13.33	93.33 ±6.67	46.67 ±6.67	73.33 ±6.67
200	100.00 ±0.00	100.00 ±0.00	80.00 ±11.55	100.00 ±0.00

Table 5. Toxicity indices and their parameters for fenitrothion and thiobencarb on earthworm by soil mixing test

Pesticide	Soil	Time (day)	LC_{50}[a] (ppm)	Confidence limits at 95%	Slope[b]	χ^2 [c]	P	Relative toxicity[d]
Fenitrothion	Clay	5	334.27	133.57-900.03	0.47 ±0.03	6.88	0.021	0.28
		10	55.45	23.86-151.71	0.35 ±0.02	8.67	0.134	0.74
	Sandy	5	415.90	198.45-933.77	0.66 ±0.07	6.62	0.342	13.29
		10	25.00	15.41-41.53	0.59 ±0.04	32.17	0.130	42.60
Thiobencarb	Clay	5	0.93	0.52-1.53	0.62 ±0.03	14.16	0.438	100
		10	0.41	0.22-0.69	0.67 ±0.04	7.91	0.491	100
	Sandy	5	55.28	34.51-91.12	0.66 ±0.04	23.71	0.214	100
		10	10.65	7.27-15.58	0.78 ±0.04	51.25	0.413	100

Note: a: Concentration causing 50% mortality. Results of LC_{50} are expressed as mean of three replicates ± standard error (SE). b: Slope of the concentration-mortality regression line ± SE. c: Chi square value. d: Relative toxicity = (LC_{50} level for the most effective pesticide/LC_{50} level for the other pesticide) × 100

In general, the acute toxicity test has been traditionally applied to assess the toxicity of soil contamination to earthworms (Fouad 2021; Fouad et al. 2023). The use of mixed pesticides is becoming increasingly popular in agriculture owing to their high efficiency, rapid action and convenience. Results from single-pesticide experiments did not actually show field situations in which multiple pesticides or pesticide mixtures are tested (Zhou et al. 2011). Therefore, more studies on the long-term effects of insecticides on earthworms are needed for adequate environmental risk assessment. Furthermore, juvenile earthworms may be more sensitive to contaminants than adults (Zhou et al. 2008). Estimating ecotoxicological risk using toxicity data from adults and single-pesticide experiments may lead to underestimation of the effects of soil pollutants on invertebrate populations. In addition, terrestrial ecosystems may not be adequately protected by the current environmental quality criteria based on exposure to a single insecticide and significantly different from those in the realistic environment, the conditions of laboratory tests are often controlled (Piola et al. 2013; Cang et al. 2017). The toxicity assays on earthworm could be used to evaluate the toxic potential of new agrochemicals or determine toxicity in contaminated soils (Fouad 2021; et al. 2022, 2023; El-Aswad et al. 2022; Shamsan et al. 2023).

In conclusion, the results of this work showed that, the concentrations of ≤ 50 μg mL^{-1} for fenitrothion and thiobencarb caused 0% mortality at 24 h. Also, both compounds fenitrothion and thiobencarb at all tested concentrations had a limited effect on earthworm *A. caliginosa* within 24 hours exposure (< 50% mortality). Therefore, LC_{50} was calculated only at 48 and 72 h. A positive correlation between the tested pesticide toxicity and exposure time or concentration was recorded. Based on the LC_{50} values, the toxicity of fenitrothion at 72 h (0.26 μg cm^{-2}) was 3.4 times more than its toxicity at 48 h (0.88 μg cm^{-2}) and the toxicity of thiobencarb at 72 h (0.42 μg cm^{-2}) was 7.2 times more its toxicity at 48 h (3.03 μg cm^{-2}). Fenitrothion was 3.5 and 1.6 times more toxic than thiobencarb at 48 and 72 h, respectively. Fenitrothion would be classified as supertoxic (LC_{50} at 48 h = 0.88 μg cm^{-2}) whereas thiobencarb would be classified as moderately toxic (LC_{50} at 48 h = 303 μg cm^{-2}). The toxicity of thiobencarb in clay soil at 5 days was 360 times and at 10 days was 135 times more than toxicity of fenitrothion. In addition, thiobencarb in sandy clay loam soil was 7.5 and 2.5 times more toxic than fenitrothion at 5 and 10 days, respectively. It should be noted that fenitrothion was least toxic to the earthworms in soil mixing test, this could be attributed to the slow degradation in the worms and subsequently less elimination of the metabolites as well as attributed to the higher adsorption and lower desorption of fenitrothion compared to thiobencarb.

REFERENCES

Aly MI, Fouad MR, Abou-Elnasr HS, El-Aswad AF. 2021. Comparison of dissipation kinetics and residual behavior for fenitrothion insecticide and thiobencarb herbicide in clay soil. Alex J Agric Sci 66: 1-11. DOI: 10.21608/alexja.2021.174541.

Araneda AD, Undurraga P, Lopez D, Saez K, Barra R. 2016. Use of earthworms as a pesticide exposure indicator in soils under conventional and organic management. Chil J Agric Res 76: 356-362. DOI: 10.4067/S0718-58392016000300014.

Badawy ME, El-Aswad AF, Aly MI, Fouad MR. 2017. Effect of different soil treatments on dissipation of chlorantraniliprole and dehydrogenase activity using experimental modeling design. Intl J Adv Res Chem Sci 4: 7-23. DOI: 10.20431/2349-0403.0412002.

Badawy MEI, Kenawy A, El-Aswad AF. 2013. Toxicity assessment of Buprofezin, Lufenuron, and Triflumuron to the earthworm *Aporrectodea caliginosa*. Intl J Zool 2013: 174523. DOI: 10.1155/2013/174523.

Beeby A. 2001. What do sentinels stand for? Environ Pollut 112: 285-298. DOI: 10.1016/S0269-7491(00)00038-5.

Cang T, Dai D, Yang G, Yu Y, Lv L, Cai L, Wang Q, Wang Y. 2017. Combined toxicity of imidacloprid and three insecticides to the earthworm, Eiseniafetida (*Annelida*, *Oligochaeta*). Environ Sci Pollut Res 24: 8722-8730. DOI 10.1007/s11356-017-8627-z.

Correia FV, Moreira JC. 2010. Effects of glyphosate and 2,4-D on earthworms (*Eisenia foetida*) in laboratory tests. Bull Environ Contam Toxicol 85: 264-268. DOI 10.1007/s00128-010-0089-7.

El-Aswad AF, Aly MI, Fouad MR, Badawy ME. 2019. Adsorption and thermodynamic parameters of chlorantraniliprole and dinotefuran on clay loam soil with difference in particle size and pH. J Environ Sci Health B 54: 475-488. DOI: 10.1080/03601234.2019.1595893.

El-Aswad AF, Fouad MR, Badawy ME, Aly MI. 2022. Effect of calcium carbonate content on potential pesticide adsorption and desorption in calcareous soil. Commun Soil Sci Plant Anal 2022. DOI: 10.1080/00103624.2022.2146131.

Fouad MR, Abou-Elnasr H, Aly MI, El-Aswad AF. 2021. Degradation kinetics and half-lives of fenitrothion and thiobencarb in the new reclaimed calcareous soil of Egypt using GC-MS. J Adv Agric Res 26: 9-19. DOI: 10.21608/jalexu.2021.165351.

Fouad MR, El-Aswad AF, Aly MI. 2022. Acute toxicity, biochemical and histological of fenitrothion and thiobencarb on fish Nile tilapia (*Oreochromis niloticus*). Nusantara bioscie 14: 217-226. DOI: 10.13057/nusbiosci/n140213.

Fouad MR, El-Aswad AF. 2018. Competitive and non-competitive adsorption of atrazine and diuron on alluvial soil. Alex Sci Exch J 39: 527-533. DOI: 10.21608/asejaiqjsae.2018.16895.

Fouad MR, Shamsan AQS, Abdel-Raheem ShAA. 2023. Toxicity of atrazine and metribuzin herbicides on earthworms (*Aporrectodea caliginosa*) by filter paper contact and soil mixing techniques. Curr Chem Lett 12: 185-192. DOI: 10.5267/j.ccl.2022.8.006.

Fouad MR. 2021. Study on toxicity effect of bispyribac-sodium herbicide on earthworms by filter paper and soil mixing method. Intl J Agric Environ Res 7: 755-766. DOI: 10.51193/IJAER.2021.7410.

Fouad MR. 2022. Validation of adsorption-desorption kinetic models for fipronil and thiamethoxam agrichemicals on three soils in Egypt. Egypt J Chem 2022. DOI: 10.21608/EJCHEM.2022.143450.6289.

Fouad MR. 2023. Physical characteristics and Freundlich model of adsorption and desorption isotherm for fipronil in six types of Egyptian soil. Curr Chem Lett 12: 207-216. DOI: 10.5267/j.ccl.2022.8.003.

Griffiths DC, Raw F, Lofty JR. 1967. The effects on soil fauna of insecticides tested against wireworms (*Agriotes* spp.) in wheat. Ann Appl Biol 60: 479-490. DOI: 10.1111/j.1744-7348.1967.tb04502.x.

Lord KA, Briggs GG, Neale MC, Manlove R. 1980. Uptake of pesticides from water and soil by earthworms. Pestic Sci 11: 401-408. DOI: 10.1002/ps.2780110406.

Lydy MJ, Linck SL. 2003. Assessing the impact of triazine herbicides on organophosphate insecticide toxicity to the earthworm *Eiseniafetida*. Arch Environ Contam Toxicol 45: 343-349. DOI: 10.1007/s00244-002-0218-y.

Martin NA. 1982. The effects of herbicides used on asparagus on the growth rate of the earthworm Allolobophoracaliginosa," in Proceedings of the 35th New Zealand Weed and Pest Conference 238-330. DOI: 10.30843/nzpp.1982.35.10579.

Mosleh YY, Ismail SMM, Ahmed MT, Ahmed YM. 2003. Comparative toxicity and biochemical responses of certain pesticides to the mature earthworm *Aporrectodea caliginosa* under laboratory conditions. Environ Toxicol 18: 338-346. DOI: 10.1002/tox.10134.

Piola L, Fuchs J, Oneto ML, Basack S, Kesten E, Casabé N. 2013. Comparative toxicity of two glyphosate-based formulations to *Eiseniaandrei*under laboratory conditions. Chemosphere 91: 545-551. DOI: 10.1016/j.chemosphere.2012.12.036.

Raj SK, Syriac EK. 2017. Herbicidal effect on the bio-indicators of soil health-A review. J Appl Nat Sci 9: 2438-2448. DOI: 10.31018/jans.v9i4.1551.

Roberts BL, Dorough HW. 1984. Relative toxicities of chemicals to the earthworm *Eisenia foetida*. Environ Toxicol Chem 3: 67-78. DOI: 10.1002/etc.5620030109.

Shamsan AQS, Fouad MR, Yacoob WARM, Abdul-Malik MA, Abdel-Raheem ShAA. 2023. Performance of a variety of treatment processes to purify wastewater in the food industry. Curr Chem Lett. DOI: 10.5267/j.ccl.2022.11.003.

Simon-Delso N, San Martin G, Bruneau E, Hautier L. 2018. Time-to-death approach to reveal chronic and cumulative toxicity of a fungicide for honeybees not revealed with the standard ten-day test. Sci Rep 8: 1-11. DOI: 10.1038/s41598-018-24746-9.

Singh V, Singh K. 2015. Toxic effect of herbicide 2,4-D on the Earthworm *Eutyphoeus waltoni* Michaelsen. Environ Process 2: 251-260. DOI 10.1007/s40710-015-0057-7.

Whitacre DM.. 2008. Reviews of Environmental Contamination and Toxicology (Vol. 202). Springer, New York. DOI 10.1007/978-0-387-79284-2.

Yasmin S, D'Souza D. 2010. Effects of pesticides on the growth and reproduction of worm: A review. Appl Environ Soil Sci 1: 9. DOI: 10.1155/2010/678360.

Zhou SP, Duan CP, Wang XH, Michelle WHG, Yu ZF, Fu F. 2008. Assessing cypermethrin-contaminated soil with three different earthworm test methods. J Environ Sci 20: 1381-1385. DOI: 10.1016/S1001-0742(08)62236-6.

Zhou SP, Duan CQ, Michelle WHG, Yang FZ, Wang XH. 2011. Individual and combined toxic effects of cypermethrin and chlorpyrifos on earthworm. J Environ Sci 23: 676-680. DOI: 10.1016/S1001-0742(10)60462-7.

ASIAN JOURNAL OF AGRICULTURE
Volume 7, Number 1, June 2023
Pages: 20-33

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g070104

Comprehensive evaluation and economic analysis in some barley genotypes under soil salinity

SAMAH A. MARIEY[1,♥], MAHA A. EL-BIALY[2], RANIA A. KHEDR[3], EMAN N. MOHAMED[4], AHMED M. I. MELEHA[2], ISMAEL A. KHATAB[5]

[1]Barley Research Department, Field Crops Research Institute, Agricultural Research Centre – Giza. 9 Gamma street, Giza, 12619 Cairo, Egypt. Tel.: +202-35693003, ♥email: samahmaraei@gmail.com
[2]Water Management Research Institute, National Water Research Center, Egypt. Al-Kanater Al-Khairia – P.O. Box 13621 Kalubia, Egypt
[3]Crop Physiology Research Department, Field Crops Research Institute, Agricultural Research Centre – Giza. 9 Gamma street, Giza, 12619 Cairo, Egypt
[4]Seed Technology Research Department, Field Crops Research Institute, Agricultural Research Centre – Giza. 9 Gamma street, Giza, 12619 Cairo, Egypt
[5]Department of Genetics, Faculty of Agriculture, Kafrelsheikh University. Qism Kafr El-Shaikh, Kafr el-Sheikh 33516, Egypt

Manuscript received: 29 October 2022. Revision accepted: 2 February 2023.

Abstract. *Mariey SA, El-Bialy MA, Khedr RA, Mohamed EN, Meleha AMI, Khatab IA. 2023. Comprehensive evaluation and economic analysis in some barley genotypes under soil salinity. Asian J Agric 7: 20-33.* Soil salinity is one of the abiotic stresses that cause a significant reduction in barley production. Understanding the phenotypic and genetic diversity among Barley genotypes is necessary to improve barley salt tolerance. Herne comprehensive sets of morph-physiological, grain quality traits and Simple Sequence Repeat (SSR) markers combined with economic analysis were done to determine the phenotypic and genetic variation of eight barley genotypes under salinity stress during seasons 2019/2020 and 2020/2021. High genetic variation was observed among studied genotypes for all measured traits. Salinization caused a significant increase in (Sodium content, soluble carbohydrate content, and crude protein content %) in sensitive genotypes (Giza 132 and line 1). SSRs markers generated clear patterns with high polymorphism with 31 alleles by an average of 2.07 alleles per locus. Out of 15 SSR markers, nine (Bmac 0209, Bmag 0011, Bmag 125, Bmac 0871, Bmag 770, Bmac 701, Bmag 0387, Bmac316, and Bmag 0009) were highly useful in distinguishing tolerant and sensitive Barley genotypes. Soil salinity decreased the benefit-cost ratio for Giza 123,136 and 137, which appear beneficial as salt-tolerant cultivars. Those cultivars had low reductions for almost studied traits and had the highest grain yield production due to increasing the farmer's income under salt affect area.

Keywords: Association analysis, barley, economic statement, parameters, phenotypic, salinity, SSR

INTRODUCTION

Crop production is mainly affected by many abiotic stresses; soil salinity is a major global climatic factor that restricts crop yields and food production worldwide (Bakari et al. 2018; Younis et al. 2020). Salt tolerance is a complex trait that uses different methods and parameters to assess plant germplasm for salt tolerance. Soil salinity is one of the chief abiotic environmental stress that causes the greatest yield losses of 20% in crop production in arid, semi-arid, coastal regions and humid and sub-humid landscapes (Mwando et al. 2020). It is estimated that around 6% of the world's total land area is affected by soil salinity, through 20% of arable land and 33% of irrigated land (Hossain 2019). Furthermore, salinity-affected areas expected to increase to be more than 50% of the world's total arable land by 2050 (Saade et al. 2016).

In Egypt, soil salinization regions were found on about 60% of the cultivated lands of the Northern Delta region, 20% of the Southern Delta and middle region, and 25% of Upper Egypt regions (FAO 2008). About 18.9 billion m^3 of wastewater was cleared directly into the Nile or into agricultural drains to be reused back to the River Nile (EEAA 2016). Salinization disturbs plants in numerous ways: water stress, nutritional disorders, and ion toxicity affecting photosynthesis. Plants' photosynthesis is one of the greatest basic biochemical and physiological processes of plant growth and productivity (Isayenkov and Maathuis 2019).

Salinity decreases the ability of plants to take up water by depressing soil water potential leading to water deficits. It disturbs all plants' growth stages through the salt-specific influence of high sodium (Na^+) and chloride (Cl^-) concentrations in tissues due to ion toxicity. The principal cause of ion-specific damage was Na^+, which greatly affects enzyme activation and protein synthesis. Salinity stress affects plant physiological mechanisms, changing plant growth, mineral dissemination, membrane insecurity, and diminished photosynthetic productivity (Acosta-Motos et al. 2017).

The best solution to the salinity problem is to use salt-tolerant species. Barley (*Hordeum vulgare* L.) consider the most salt-tolerant species among cereal. It ranks the fourth important cereal crop as a major food source and feeds many animals and people living in complicated areas. Due to its resilience and relatively stable yields, under both highly productive areas and subsistence low-input agricultural systems compared with other grains crops (FAOSTAT 2019). It is an excellent model crop for studies of mechanisms and inheritance of salinity tolerance which using in developing tools to improve salt tolerance in cereals (Zhu et al. 2020). A major objective of crop

breeding programs is to produce new varieties with improving responses to salt tolerance and to get high yields through evaluating diverse genotypes to increase their effectiveness (Allel et al. 2019; Al-Ashkar et al. 2020; Akhter et al. 2021; Mariey et al. 2022a,b).

Barley breeders are working to understand the behavior and efficiency of salinity on barley plants. Several researchers in barley have studied the effect of salinity stress on agro-physiological and biochemical traits (Mariey et al. 2017; Mariey et al. 2018; Mariey et al. 2019; Rajeswari et al. 2019; El Sabagh et al. 2019; Sorkhi 2020; Zeeshan et al. 2020; Akhter et al. 2021; Dell'Aversana et al. 2021; Mariey et al. 2022a,b) which they reported that salinity stress harmfully affects practically all stages of plant growth and development.

DNA based markers are required methods that were not influenced by environmental conditions. Therefore, it is consistently used for polymorphism, fingerprinting, genetic diversity detection, and genetic analyses (Grover and Sharma 2016). Among several types of DNA molecular markers applied in different genetic studies, microsatellites or Simple Sequence Repeats (SSRs) were still actually used in breeding programs. Those markers provide important information about a species, as they have a high polymorphism rate, co-dominant inheritance, high reproducibility, locus specificity, and random distribution on the genome (Brbaklic et al. 2021). Moreover, SSRs are powerful markers that are excellent for assessing genetic diversity and crop improvement for salinity stress tolerance (Mariey et al. 2016; Khatab et al. 2021; Mehta et al. 2021; Mariey et al. 2022a).

Hence, successful salinity tolerance breeding programs must adopt a strategy to identify salt-tolerant genotypes by identifying comprehensive donating characteristics such as physiological, morphological, and biochemical traits. Therefore, metabolic pathways with molecular markers recognize their contribution to salt tolerance to improve and increase crop yields in saline areas to produce enough food for the increasing global population (Mwando et al. 2020; Khatab et al. 2021). So, the association of molecular markers with phenotypic evaluation is an important factor in investigating tolerance's genetic role by predicting the genomic regions that affect plant response. Those will be useful as a comprehensive evaluation of breeding programs for environmental stress (Sallam et al. 2018; Khatab et al. 2021; Mariey et al. 2021; Mariey et al. 2022b).

Even though increasing crop production per unit under saline areas is necessary, an increase in the farmer's income is the most important goal because of the non-linear between crop yield and the price of products. Moreover, soil salinity affected negatively on crop growth and productivity, which leads to lower revenues, profitability, and land values, so knowing the crop that should be grown in saline lands is an important part of the on-farm decision makers (NDSU 2015; Hammami et al. 2020).

Thus, the present study aimed to comprehensively evaluate relative importance traits such as morph-physiological, grain composition parameters, and SSR analysis combined with economic analysis to accurately determine the phenotypic and genetic diversity of eight Barley genotypes. This study would also establish specific markers traits associated with salt tolerance to categorize Barley genotypes to use them in salinity breeding programs and to increase the production in the saline area in Egypt.

MATERIALS AND METHODS

Barley genotypes

Eight Egyptian barley genotypes kindly provided by Sakha Barley Research Department, Field Crops Research Institute, Agricultural Research Center, Egypt, are used in this study. First, those barley genotypes were observed to know their response to salinity stress as a first step to using them in the barley breeding program for salinity. Next, through crossing between them as a second step, names, rows-type, pedigree, salinity response, and years were shown in (Table 1).

Field experiments

Field experiments site

Two environmental field experiments were conducted under saline-sodic and normal conditions during two growing seasons, 2019/20 and 2020/21, at El Karada Water Requirements Research Station farm, Water Management Research Institute, National Water Research Center, in Kafr El-Sheikh Gov, Egypt. That site is located at 31° 05' 36.28" N, 30° 56' 53.56" E, with an elevation of 6 meters above mean sea level.

Table 1. Name, row type, salinity response pedigree, and released years of eight barley cultivars used in the field experimental

Name	Row type	Pedigree	Salinity response	Released year
Giza 123	Hulled	Giza 117/FAO 86	Tolerance	1998
Giza132	Hulled	Rihane-05//AS 46/Aths*2Athe/ Lignee 686	Sensitive	2006
Giza 136	Hulless	Plaisant/7/cln-b/ligee640/3/s.p-b//gloriaar/ come b/5/falconbar/6/linocln-b/a/s.p-/lignee640/3/s.p-b//gloria-bar/come b/5/falconbar/6/lino	Tolerance	2011
Giza 137	Hulled	Giza 118 /4/Rhn-03/3/Mr25-//Att//Mari/Aths*3-02	Un-know	2017
Giza 138	Hulled	Acsad1164/3/Mari/Aths*2//M-Att-73-337-1/5/Aths/ lignee686 /3/Deir Alla 106//Sv.Asa/ Attiki /4/Cen/Bglo."S")	Un-know	2017
Promising Line1	Hulled	C .C 89/3/Alanda/Hamra//Alanda-01	Un-know	-
Promising Line2	Hulled	Giza 118/3/Alanda/Hamra//Alanda-01	Un-know	-
Promising Line3	Hulled	Giza 124/6/Alanda//Lignee527/Arar/5/Ager//Api/CM67/3/ Cel/WI2269//Ore/4/ Hamra-01	Un-know	-

Field experiments design

Barley genotypes were planted in a Randomized Complete Block Design (RCBD) with three replicates; each plot was devoted to one genotype, which was planted in four rows 2 m long, spread out with 20 cm among rows (plot area= 1.6 m^2).

Sowing was done at a rate of 210 kg ha^{-1} using the broadcasting method on 23 and 25 November 2019, the 2020 seasons. Harvesting on 8 and 10 May 2020, the 2021 seasons. The normal cultural practices for growing barley were applied as recommended according to the Ministry of Agriculture's recommends.

Field experiments of site soil samples

Soil samples were taken before land preparation from the experimental site to a depth of 30 cm from the soil surface to perform physical and chemical analyses, as presented in Table 2.

Field experiments of site crop evapotranspiration

Crop evapotranspiration under normal soil (ETc, mm/day). Etc is evaporation from perfectly managed fields, large, well-irrigated that give full production under available climatic conditions is given by the equation below.

$$ETc = kc \times ET0 \quad (1)$$

Where: Kc is crop coefficient, and ETo is Reference crop evapotranspiration (mm/day^{-1})

Crop evapotranspiration under saline soil conditions (Etc adj). Etc adj is the evapotranspiration from crops cultivated under environmental management conditions that deviate from the standard circumstances.

$$ETc\ adj = ks \times kc \times ET0 \quad (2)$$

Where, ks is the water stress coefficient measured phenotypic characters affected by salinity stress. Under both control and saline condition, the following phenotypic characters were measured for each genotype in the two growing seasons; the types of traits, unite, abbreviation, and methodology reference of each trait are shown in Table 3.

Table 2. The average of physical and chemical properties and soil classification for soil samples from the field experiments sites during two growing seasons 2019/2020 and 2020/2021

Soil analysis		Normal site	Saline site
Physical analysis			
Soil texture	Coarse sand (%)	1.56	2.04
	Fine sand (%)	18.94	18
	Silt (%)	28.15	27.6
	Clay (%)	51.35	52.36
	Texture	Clayey	Clayey
Soil moisture	Bulk density (g cm^{-3})	1.21	1.15
Constants	Field capacity (%)	40	39.9
	Wilting point (%)	21.7	20.5
Chemical analysis			
EC(dSm-1)		2.76	10.7
PH		7.6	7.8
Sodium Absorpation Ratio SAR		2.63	17.84
Exchangeable soduim percentage ESP		10.11	45.50
Soluble cations	Ca++	0.33	7.82
meq100 1 g soil	Mg++	0.15	28.58
	Na+	1.29	76.1
	K+	0.1	2.05
Soluble anions	$CaCO^{3-}$	0	0
meq100-1 g soil	HCO^{3-}	13.2	59.9
	Cl^-	23.35	33
	SO^{4--}	4.95	14.1
Soil classification		Non-saline	Saline-sodic soils

Table 3. The names, units, abbreviations, and methodology references of all traits

Types of traits	Traits	Unite	Abb.	Methodology reference
Physiological traits	Chlorophyll a	µg mL^{-1}	Ch. a	(Moran 1982)
	Chlorophyll b	µg mL^{-1}	Ch. b	(Moran 1982)
	Relative water content	%	RWC	(Gonzalez and Gonzalez-Vilar 2001)
	Sodium Na content	mg g^{-1}Dw	Na	(Chapman and Pratt 1978)
	Potassium K content	mg g^{-1}Dw	K	(Chapman and Pratt 1978)
	Soluble carbohydrate content	mg g^{-1}Dw	**SCC**	(Naguib 1962)
Agro-morphological traits	Days to heading	days	HD	Days from sowing to 50% flowering
	Plant height	cm	PH	length of plant from soil to tip spike
	Number of grains $spike^{-1}$	grains	NGS	Number of grains each spike
	Number of tillers m^{-2}	tillers	NT	Number of tillers per m^2
	Thousand kernel weight	g	TKW	Weight 1000 grain
	Grain yield	t ha^{-1}	GY	Weight grain per plot
Grain composition traits	Crude protein content,	%	CPC	(Peter and Young 1980)
	Total ash content	%	TAC	(AOAC 2000)
	Total starch content	%	TSC	(Duis et al. 1956)
	Crude fiber content	%	CFC	(AOAC 2000)

Physiological traits

All the physiological traits done at the heading stage of barley leaves from the top of ten plants from each plot were randomLy taken to determine the physiological traits as follows: Photosynthetic pigments (chlorophyll a (Chl a) and chlorophyll b (Chl b)) were determined with approximate ratios of 1:100 (w/v) for fresh leaves. While N, N-dimethylformamide and determined spectrophotometrically at two wavelengths (664 and 647), according to Moran (1982).

$$Cha = 12.64\ A664\text{-}2.99A647 \quad (3)$$
$$Chb = \text{-}5.6\ A664 + 23.26\ A647 \quad (4)$$

Where: A664: the absorbance at wavelength 664; A647: the absorbance at wavelength 647

Relative Water Content (RWC%) was measured according to (Gonzalez and Gonzalez-Vilar 2001). Fresh leaves were cut into small pieces and weighed as Fresh Weight (FW), then sodden for 24 h in distilled water and weighed again to obtain the Turgid Weight (TW). Then, they were dried and weighed to obtain DW then using the equation.

$$RWC = (FW - DW/TW - DW) \times 100 \quad (5)$$

Where: FW is fresh weight, DW is dry weight, and TW is the turgor weight of leaf samples.

Sodium (Na) and potassium (K) content (mg g-1DW) were measured on the dry sample using standard flame photometer procedure (Model PFP7 Flame photometer, Jenway, Bibby Scientific Ltd, UK) as described by (Chapman and Pratt 1978) and soluble carbohydrate content SCC ($mgg^{-1}DW$) was measured on dry sample according to (Naguib 1962).

Agro-morphological traits

Six agro-morphological traits were estimated to study the effect of salinity on morphological traits and yield and its compound traits. (i) Days to Heading (HD): measured as the number of days to flowering when 50% of the spikes in a plot had extruded anthers. (ii) Plant Height (PH): was measured on a random sample of five plants in each plot as the length from the soil surface to the tip of the spike at harvest time. (iii) Number of Grains per Spike (NGS^{-1}): was measured on a random sample of ten spikes in each plot as calculated by the mean of grain number. (iv) Number of Tillers per m^2 (NT m^2): was measured on a random sample of ten spikes in each plot as calculated the mean of tillers number for each plot. (v) Thousand Kernel Weight (TKW): was measured by weighing 1000 kernels randomLy from each genotype grain yield. (vi) The Grain Yield (GY): was determined by harvesting the yield of the central area (1.6 m^2) of the plot and then transformed to the unit of ($t\ ha^{-1}$)

Grain composition traits

Harvested grains of the eight Barley genotypes from two seasons were composed and bulked per each genotype and grounded to a fine powder to measure: (i) Crude Protein Content (CPC%), the grain nitrogen percentage was determined using the micro-Kjeldahl method as described by Peter and Young (1980). (ii) Total Ash Contents (TAC%) were measured according to (AOAC 2000). (iii) Total Starch Content (TSC%) which was determined by the sulfuric phenol method (Duis et al. 1956). (iv) Crude Fibers Content (CFC %) was determined according to (AOAC 2000).

DNA extraction and SSR-PCR Reaction

DNA extraction

Total genomic DNA was extracted from young fresh leaves of eight barley genotypes CTAB protocol according to Doyle and Doyle (1990). DNA quality and concentration were estimated using NanoDrop Spectrophotometer (ND-1000, Thermo-Scientific).

Microsatellite primer

Fifteen Microsatellite SSR primer pairs were previously mapped and covered. All seven barely chromosomes (Grain Genes database) were selected from the published genetic maps according to (Varshney et al. 2007) against eight Barley genotypes to identify their polymorphic markers were shown in (Table 4).

Table 4. The name, sequence, and chromosome location of 15 SSR primers

Primer name	Sequence		Chromosome location
Bmag770	F:AAGCTCTTTCTTGTATTCGTG	R:GTCCATACTCTTTAACATCCG	1H
Bmac0213	F: ATGGATGCAAGACCAAAC	R:CTATGAGAGGTAGAGCAGCC	1H
Bmag749	F:CGGATTCTTGAGTAGTCTCTG	R:GATCTGTTTTTTGTAGAACATGC	2H
Bmag0125	F: AATTAGCGAGAACAAAATCAC	R:AGATAACGATGCACCAC	2H
Bmac0209	F:CTAGCAACTTCCCAACCGAC	R:ATGCCTGTGTGTGGACCAT	3H
EBmac0871	F:TGCCTCTGTTGTGTTATTGT	R: CCCCAAGTGAACATTGAC	3H
EBmac0701	F:ATGATGAGAACTCTTCACCC	R:TGGCACTAAAGCAAAAGAC	4H
HVMLOH1A	F:CCTCCCCTCTGATATGATAA	R:GTACAGACGGTTTAATTGTCC	4H
Bmac0113	F:TCAAAAGCCGGTCTAATGCT	R:GTGCAAAGAAAATGCACAGATA	5H
Bmag0387	F:CGATGACCATTGTATTGAAG	R:CTCATGTTGATGTGTGGTTAG	5H
Bmac0316	F':ATGGTAGAGGTCCCAACTG	R :ATCACTGCTGTGCCTAGC	6H
Bmag0009	F:AAGTGAAGCAAGCAAACAAACA	R:ATCCTTCCATATTTTGATTAGGCA	6H
Bmag0011	F:ACAAAAAACACCGCAAAGAAGA	R:GCTAGTACCTAGATGACCCCC	7H
Bmag0135	FACGAAAGAGTTACAACGGATA	R:GTTTACCACAGATCTACAGGTG	7H
EBmac0603	F:ACCGAAACTAAATGAACTACTTCG	R:TGCAAACTGTGCTATTAAGGG	7H

PCR-SSR reaction

SSR reaction was carried out in 25 µL using 20 ng/µL of genomic DNA templates. Next, 2.5 µL of 1X PCR buffer containing (15 mM MgCl, 0.5 µL of 15 mM dNTP mixture (2.5 mM of each), 1.25 µL of 5 u/µL of *Taq* DNA polymerase, and 0.25 µL of 10 µM forward and reverse primers) and18.5 ddH2O for amplification.

PCR amplification

PCR was carried out as the following PCR program; initial denaturation at 94°C for 5 min for one cycle, denaturation at 95°C for 1 min for 35 cycles, and annealing temperature (45-55°C) are specific for each primer for 30 sec and final extension at 72°C for 7 min with final holding at 4°C.

Gel electrophoresis

Amplified products were separated using agarose gel electrophoresis (2%) in 0.5 x TBE buffer against 100 bp DNA Ladder.

Economic impact statement

The economic productivity of eight barley genotypes was measured through Total Seasonal Return (TSR) ($/ha), Total Costs (TC) ($/ha), Net Return (NR) ($/ha), and Benefit-Cost Ratio (BCR) as affected by soil salinity were expressed in productive crop units of kg/m^3, and price of barley grains (1.5 $ per 1 Kg) for each treatment were done according to (Cimmyt 1988).

Data analysis

Phenotypic variation data analysis

A test for homogeneity of variance was used to compare variances over two years before determining the cogency of combined analysis according to the Bartlett test (Bartlett 1937).

All the data were statistically exposed to ANOVA in a two-way Randomized Complete Block Design (RCBD) to conclude the effects of genotypes, salinity, and their interaction on the studied traits, performed using SPSS-16.0 statistical software package (SPSS Inc. Chicago, IL, USA). The means of the different phenotypic parameters were compared by the Least Significant Difference (LSD). Correlation analysis using Pearson's parametric correlation test was performed using SPSS 22.0 (SPPS Inc. Chicago, IL) to determine the relationship between the two traits studied. GGE-biplots were used to study genotype-by-environment interaction using the Principal Component Analysis (PCA) to display the two-way data in the bi-plot graph. These were performed using a computer program Minitab v. 19 (Minitab Inc Coventry, UK), according to Sally et al. (1986). ClustVis, a web tool for visualizing the clustering of multivariate data, was used to construct heatmaps (Metsalu and Vilo 2015).

Molecular markers data analysis

The amplified bands from SSR were scored as binary data under the heading of total scorable fragments, which were determined for each genotype. The data were used to calculate allele frequencies, allele number, and genetic similarity according to (Varshney et al. 2007). Polymorphism Information Content (PIC) values were estimated according to (Nei and Li 1979). The marker efficiency of the 15 SSR primers, including diversity index (DI), Effective Multiplex Ratio (EMR), Resolving power (Rp), discriminating power, and Marker Index (MI) values, were calculated according to (Amiryousefi et al. 2018). Un-weighted Pair-Group Method with Arithmetical (UPGMA) cluster analysis was performed to produce a dendrogram on Jaccard's similarity coefficient using the PAST (Paleontological Statistics) software package (Hammer et al. 2001).

RESULTS AND DISCUSSION

Effect of salinity on the experimental soil

The variability of biophysical environments on experimental sites

The field experiments were conducted in two contrasting biophysical environments. The soil of the first environment is classified as non-saline soil, containing salts that provide an ECe of 2.76 dSm^{-1} and ESP of 10.11% and are very diverse, from soil rich in clay and poor in organic matter. While the second soil of the other environment is classified as Saline-Sodic soil, which contains salts that provide an ECe of 10.7 dSm^{-1} and ESP of more than 15, i.e.(45.5%), which leaching is difficult for the reason that the clay colloids are dispersed as shown in Table 2.

The changeability of soil salinity on crop evapotranspiration (ETc and Etc adj)

Crop growth season has been divided into four growth stages: initial, development, mid-season, and late season, as shown in Figure 1. The water needs of barley were calculated during the growth period by multiplying the evaporation and crop coefficient for each of the four stages of growth. The results showed the accumulative values of ETc were 170.30 and 141.35 mm for the two types of abovementioned soils, respectively distributed as follows: 16.94, 35.68, 89.93 and 27.75 mm and 14.06, 29.62, 74.64 and 23.03 at various stages of growth respectively. Additionally, the ETc was 17% higher in non-saline soil than in saline-sodic soil.

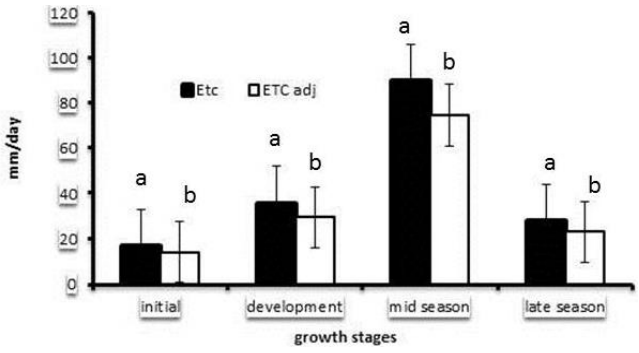


Figure 1. The effect of soil salinity on crop evapotranspiration (ETc and Etc adj)

Influence of salinity stress on the phenotypic traits among barley genotypes

Analysis of variance

A combined analysis of variance for the sixteen different phenotypic traits with homogeneous variance across the two seasons was measured on the eight Barley genotypes presented in Table 5. According to the Fisher variance comparison test for all measured phenotypic parameters, highly significant differences in treatments (T) (between normal and salinity stress) were found. ANOVA analysis revealed highly significant differences among genotypes (G) in all studied traits. The genotype × treatment interaction significantly differed among all studied traits, except total starch content TSC was non-significant.

The means performance of barley genotypes due to salinity stress

Physiological traits

Salinity stress caused a significant decrease in photosynthetic pigments Cha (Chlorophyll a) contents in all the eight Barley genotypes compared to its content under normal conditions by average reduction values (44.07%). The highest Cha content was found in Giza 137, with a value of 11.99 µg mL^{-1} under normal conditions, and Giza 123 had the maximum value (8.71 µg mL^{-1}) under salinity conditions. While Line 1 had the lowest Cha content under normal soil (8.47 µg mL^{-1}), and Line 3 recorded the lowest one (3.38 µg mL^{-1}) with an average value (5.71) under the salt condition as shown in (Figure 2A, Table 6).

In the case of Chb (Chlorophyll b) content, salinity decreased Chb content in all genotypes by average reduction values (54.46%). Giza 137 had the highest Chb content with a value (of 5.03µg mL^{-1}) under the normal condition with an average value (of 4.02), and Giza 136 had the highest Chb (2.83 µg mL^{-1}) under the salinity condition. However, Line 3 had the lowest Chb content (3.18 µg mL^{-1}) under normal conditions, while Line 1 gave the lowest Chb content (1.59µg mL^{-1}) under salt stress, as shown in (Fig2B, Table 6). Similar results were obtained by (Lin et al. 2016; Zeeshan et al. 2020; Akhter et al. 2021). They claimed that both chlorophyll a and b contents of barley plant decreased in response to salinity stress due to the rise of chlorophyllase enzyme, which converts chlorophyll into chlorophyllide and python under saline conditions.

Salinity stress significantly reduced RWC by 5.23 % in all eight Barley genotypes. Results (Figure 2C, Table 6) showed that high RWC was found in Giza 137 under normal and salt stress (84.25 and 80.84%), respectively. On the other hand, line 1 had the lowest RWC percent (62.17 and 60.08%) under both normal and salinity stress, respectively. Similar results were obtained with (Dell'Aversana et al. 2021), who reported that RWC is an important trait, reflecting the ability of plants to adjust their osmotic potential to preserve turgor pressure under salinity stress. Thus, the genotype with a high RWC percentage can tolerate salinity stress. In this study, Giza 137 recorded the highest RWC percentage while Line1 had the lowest percentage.

Salinity stress caused increasing in Na content in all Barley genotypes under salinity conditions as compared to the normal condition with 43.87%. However, these increases differed greatly among the genotypes (Table 6, Figure 3D). Line 1 had the highest Na content with values (of 0.99 and 2.023 mgg^{-1}DW) under normal and salinity conditions, respectively. While Giza 138 had the lowest Na content (0.736 mgg^{-1} DW) under normal treatment and Giza 123 (1.26 mgg^{-1}DW) under salinity treatment.

In the case of K content, salinity significantly reduced K content by (26.71%), in all genotypes (Table 6, Figure 2E). Giza 136 had high K content (2.63 and 2.15 mg g^{-1} DW) under normal and salinity treatment, respectively. While Line 1 had the lowest K content (1.79 and 1.14 mg g^{-1}DW) under normal and salinity treatments, respectively. The results agree with (Mahlooji et al. 2018; Isayenkov and Maathuis 2019; Narimani et al. 2020; Sorkhi 2020; Zhu et al. 2020). They found that the accumulation of toxic Na^+ in cytoplasm motivates stomatal closure, which causes a strong imbalance between entered light and energy utilization, decreases the photosynthetic rate and lead to the formation of Reactive Oxygen Species (ROS). Excessive Cl^- and Na^+ uptake leads to Ca^{2+} and K^+ deficiency and other nutrient imbalances. Tolerant genotypes of barley may avoid Na^+ accumulation in aboveground parts, which facilitates the photosynthetic process and leads to higher grain yield and has minimum Na content and maximum K content.

Salinity significantly increased Soluble Carbohydrate Content (SCC) by 6.79% among all studied genotypes (Table 5, Figure 2F). Giza 132 had the maximum SCC under normal and salinity stress (0.40 and 0.44 mgg^{-1} DW). While the minimum SCC (0.31 and 0.33 mgg^{-1} DW) was found in line 3 under normal and saline conditions, respectively. Similar results were observed in barley, where water deficit is the first effect of salinity stress on plants, and the accumulation of soluble sugars in leaves is a way for osmotic equipoise when plants are exposed to salt stress (Narimani et al. 2021).

Agro-morphological traits

Salinity stress quickens days to heading (HD) with high significant differences among Barley genotypes, through average values of (87.29 and 80.5 days) under salinity and normal condition, respectively, as shown in (Figure 3A, Table 6). The results exhibited that Giza 136 was the earliest cultivar at normal and salinity conditions (77.91 and 81.13 days), respectively. While Line 1 was the latest Barley genotype with values (84.47 and 91.80 days) under normal and salt stress, respectively. The same results were comforted by (Saade et al. 2016; Mariey et al. 2017; Allel et al. 2019; Mariey et al. 2022b), who identified that there is the genetic basis for earlier flowering in Barley genotypes associated with higher salinity tolerance.

Table 5. The analysis of variance for physiological, agro-morphological, and grain quality traits of eight Barley genotypes combined over the two seasons

Trait	Genotypes (G)	Treatments (T)	GXT	LSD
Physiological traits				
Chlorophyll a	15.06**	224.5**	3.86*	0.001
Chlorophyll b	1.406**	39.54**	0.283*	0.061
Relative water content	92.05**	0.300**	0.800*	0.115
Sodium Na+ content	0.023**	0.166**	0.017*	0.203
Potassium K+ content	0.316**	2.075**	0.112*	0.081
Soluble Carbohydrate Content	0.004**	0.0162**	0.0013*	0,004
Agro-morphological traits				
Days to Heading	55.015**	351.7**	19.142**	0.502
Plant Height	2789**	7268**	134.1*	0.678
Number of grains spike^{-1}	1440**	1816.7**	97.55**	0.553
Number of tillers m	11918**	376518**	3414.8*	0.319
Thousand kernel weight	4.309**	13.44**	0.457**	0.153
Grain Yield	3.93**	75.33**	0.177*	0.167
Grain chemical composition				
Crude protein content	0.504**	40.16**	1.534**	0.20
Total Ash content	0.1237**	4.057**	0.078*	0.001
Total starch content	7.56**	229.6*	1.27 NS	1.32
Crude fiber content	2.1104**	14,85**	0.2118*	1.07

Note: Ns, * and ** are non-significant and significant at the 0.05 and 0.01 levels of probability, respectively

Table 6. Minimum (Min.), maximum (Max.), an average of each trait scored on the Barley genotypes under normal and salinity stress

Traits	Normal condition			Salinity stress condition		
	Min.	Max.	average	Min.	Max.	average
Physiological traits						
Chlorophyll a	8.47	11.99	10.04	3.38	8.71	5.71
Chlorophyll b	3.18	5.03	4.02	1.68	2.83	2.20
Relative water content	62.17	84.25	75.78	60.08	80.84	71.05
Sodium Na content	0.74	0.99	0.93	1.33	2.023	1.52
Potassium K content	1.79	2.63	2.14	1.14	2.15	1.75
Soluble carbohydrate content	0.31	0.40	0.36	0.35	0.44	0.40
Agro-morphological traits						
Days to Heading	77.91	84.47	80.51	81.13	91.80	87.29
Plant height	99.80	117.13	105.70	71.80	91.47	81.09
Number of grains spike-1	42.13	96.13	68.13	36.47	72.47	55.83
Number of tillers m-2	286.80	466.80	369.47	140.33	233.47	192.34
Thousand kernel weight	8.46	11.99	10.04	3.41	7.81	5.59
Grain yield	3.94	5.97	5.11	1.36	3.82	2.55
Grain chemical composition						
Crude protein content	10.21	12.43	11.55	12.52	14.39	13.38
Total Ash content	2.38	3.02	2.78	1.88	2.19	2.26
Total starch content	60.41	64.39	62.34	55.39	62.34	57.88
Crude fiber content	4.97	6.87	5.73	3.97	5.73	4.72

Salinity stress caused a significant decrease in the plant height PH among all genotypes, as shown in (Table 6, Figure 3B). The Egyptian cultivar Giza 137 had the tallest genotypes with 117.13 and 91.47 cm values under both normal and saline conditions, respectively. Conversely, Line 1 displays the shortest genotypes under normal and salt conditions with values of 99.8 cm and 71.8 cm, respectively. Salt stress harmfully affected the Number of Grain Spike-1, (NGS) by an average reduction of 17.26%, as shown (Table 6, Figure 3C). The Egyptian cultivar Giza 137 had the highest NGS under normal and salt-stressed conditions, with 96.13 and 72.47 NGS values. At the same time, the lowest NGS was shown by line 1 with average values of (68.13 and 55.83) under normal and salt-stressed conditions, respectively. Soil salt stress reduced the Number of Tillers m^{-2} (NT) for all genotypes once compared with normal conditions. Results showed that Giza 123 and Giza 137 had the highest NT under normal and salt-stressed conditions. In contrast, the lowest NT was shown by line 1 under the normal condition with average values of (394.47 NTm^{-2}), and Giza 138 with average values of (192.39 NTm^{-2}) under salt-stressed conditions (Table 6, Figure 3D). Soil salinity decreased in Thousand Kernels Weight (TKW) in all genotypes (Table 6, Figure 2E). The maximum TWK was produced by Giza 137 under normal and salinity stress with values (of 11.99 and 7.81 g), respectively. While the minimum TWK was exhibited by line 1 and Giza 132 with average (10.04 g and 5.59 g), respectively, under normal and salinity.

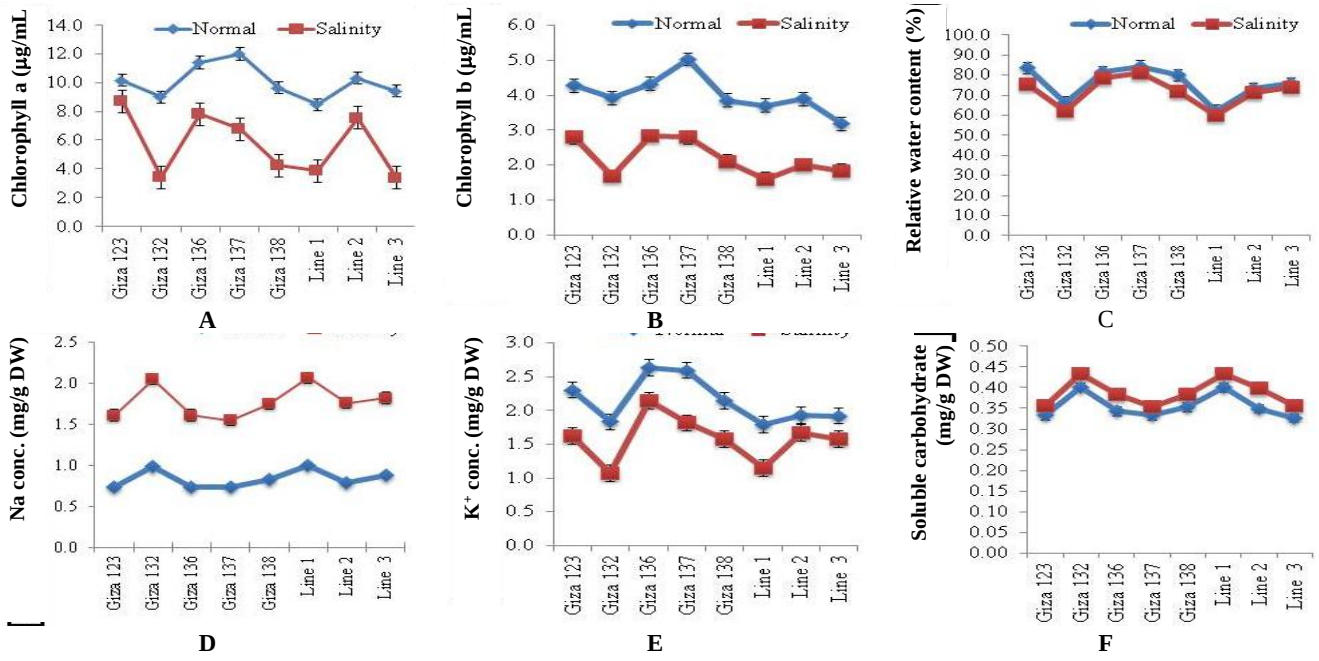


Figure 2. Effect of salinity stress on physiological traits in the eight barley genotype

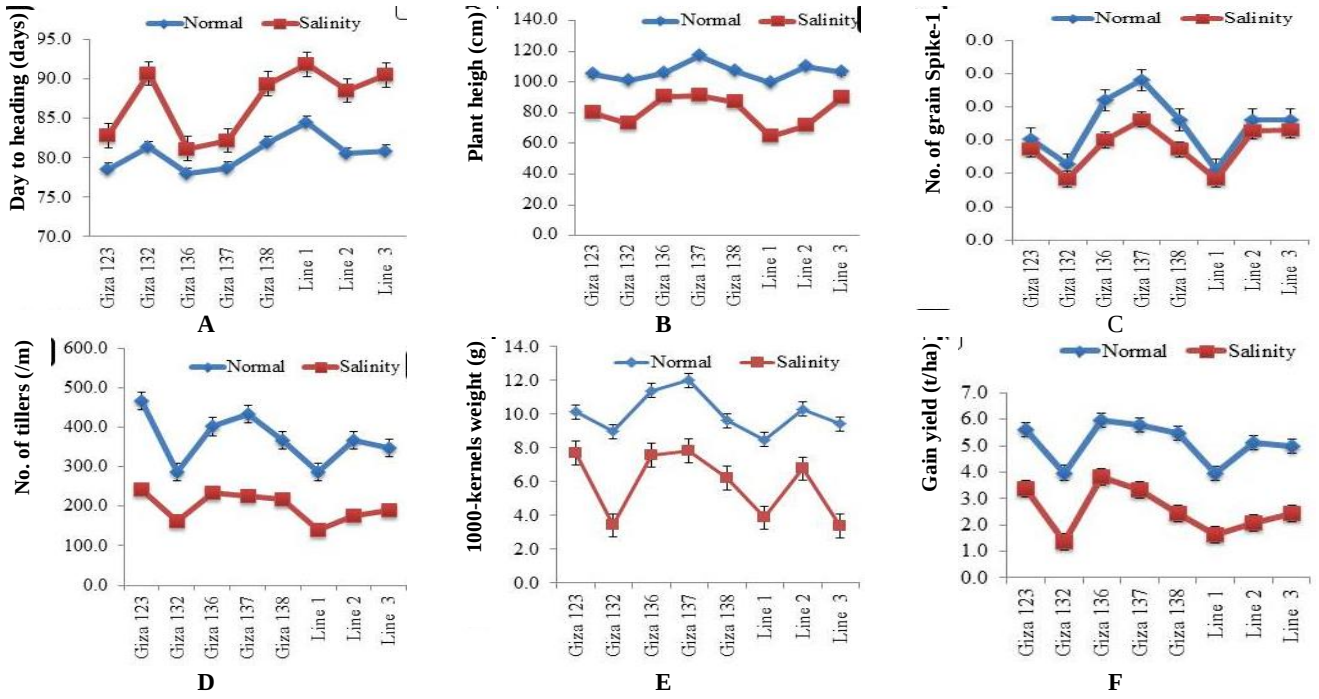


Figure 3. Effect of salinity stress on agro-morphological traits in the eight Barley genotype

In addition, salt stress dangerously affected grain yield GY (t ha^{-1}) by a high average reduction of 51.19%. Salinity caused a decrease in GY for all genotypes under study with an average (5.11 and 2.55 t ha^{-1}) under normal and salinity stress, respectively, as presented (Table 6, Figure 3F). Results showed that Giza 136, Giza 137, and Giza 123 had the maximum GY values (5.97, 5.79, and 5.59 t ha$^{-1)}$ under normal conditions and (3.82, 3.37, and 3.36 t ha^{-1}) under stress conditions. However, line 2 and Giza 132 had the minimum values under normal and stress conditions. Similar results have been observed by (Mariey et al. 2018; El Sabagh et al. 2019; Mariey et al. 2019; Rajeswari et al. 2019; Zeeshan et al. 2020; Akhter et al. 2021; Dell'Aversana 2021; Mariey et al. 2022a,b), they confirmed that the reduction in grain yield was due to reduction in yield components. Grain yield is a final product of the action and interaction of many environmental, agronomical, and physiological characteristics.

Grain composition traits

Salt stress significantly increased Grain Protein Content (GPC) in all genotypes under salinity stress more than in normal conditions (Table 6, Figure 4A). For example, under salinity stress, Giza 132 had the maximum GPC (14.39 %) in line 3, and line 1 had the minimum GPC (12.83%). Under normal conditions, Giza 137 had the maximum GPC with values (of 12.43%), while line 1 had the minimum GPC (10.21%) with an average (of 11.55%). Our results are in good harmony with those (Saleh-Amal et al. 2017; Nadeem et al. 2020), who reported that salinity increases CPC. This high protein might play an important role in increasing the osmotic pressure of the cytoplasm under salt stress and reducing other grain compositions.

However, salinity stress reduced total ash content TAC in all eight Barley genotypes, as displayed in (Table 5, Figure 4B). Giza 137 and Giza 123 had the same highest TAC under normal and salinity conditions with values of (3.02 and 2.38 $mgg^{-1}Dw$), respectively. Results showed that Line 1 had the lowest TAC content under normal and salinity with an average (of 2.78 and 2.19%), respectively.

Moreover, salt stress reduced Total Starch Content (TSC) by an average reduction of 7.82% in all the studied genotypes (Table 6, Figure 4C). The maximum value of TSC was found in Giza137 with values (of 64.39%) under normal, and Giza 136 was (59.37%) under stress conditions. While line1 had the minimum values of TSC under normal and salinity stress.

In addition, salinity stress decreased crude fiber content CFC in all eight Barley genotypes with an average decrease (5.73 and 4.62%) under normal, and Salinity conditions, respectively (Table 6, Figure 4D). Giza 136 and Giza 137 both had the maximum value of CFC. At the same time, Giza 132 and line 1 recorded the minimum values of CFC under normal and salinity, respectively.

Pearson correlation analysis

The Pearson correlation between all sixteen studied parameters under two different soil salinity levels over two seasons is displayed in Figure 5. Under normal conditions, GY displayed a highly significant and significant positive correlation with Cha, PH, NGS, NT, CPC, RWC, K^+, and TSC. While it had a significant negative correlation with Na^+, SCC, and HD**.** However, under salinity stress, GY had a highly positive correlation with Cha, K^+, RWC, PH, NGS, and NT, while GY had a significant negative correlation with Na^+, SCC, and HD. The phenotypic correlation between normal and salinity stress conditions for the same trait was exposed in (Figure 6, yellow cells.). A strong positive correlation ($|r|> 0.7$) was found in six traits (Cha, RWC, Na^+, NGS, NT, and GY, and a medium positive correlation ($|r|> 0.5$) was found in six traits (Cha, K+, SCC, HD, PH, and NT) while (TAC, TSC, and CFC) exhibit negative correlation.

Genotypic diversity among eight Barley genotypes due to salinity stress

SSR marker efficiency indices analysis

Thirty-one alleles were generated from 15 SSR primers using studied eight Barley genotypes with an average of 2.07 alleles per locus (Table 7). Five SSR primers revealed monomorphic fragment profiles as one marker were Bmac 0213 (1H), Bmag749(2H), HVMLOHIA (4H), Bmac 0113 (5H), and Bmag0135 (7H). Five SSR primers produced two fragments, Bmac 0209 (3H), Bmac 0871 (3H), Bmag 0009 (6H), Bmag 0011 (7H), and EBmac 0603 (7H). Four SSR primers produced three bands, Bmag 125(2H), EBmac 0701(4H), Bmag 0387 (5H), and Bmac 316 (6H), besides the SSR primer Bmag 770 (1H) primer produced four fragments.

Table 7. The marker efficiency indices of multiplexing sets of the used 15 SSR primers

Primer name	Motifs	Size band	NA	NPB	PIC	PP%	DI	EMR	DR	PR	MI
Bmag770	(GT)13 (AG)19	158	4	4	0.85	100	0.49	2.6	0.57	2.75	0.087
Bmac0213	(AC)23	168	1	0	0	0	0	1.0	0	0	0
Bmag749	(AG)11	166	1	0	0	0	0	1.0	0	0	0
Bmag0125	(AG)19	134	3	2	0.37	66.7	0.46	1.12	0.86	1.75	0.021
Bmac0209	(AC)13	176	2	2	0.47	100	0.48	1.25	0.62	1.0	0.036
EBmac0871	(TG)13	180	2	1	0.51	50	0.49	1.13	0.70	1.75	0.034
EBmac0701	(AC) 23	149	3	3	0.48	100	0.38	2.5	0.44	1.50	0.035
HVMLOH1A	(GA)6	175	1	0	0	0	0	1.0	0	0	0
Bmac0113	(AT)7 (AC)18	187	1	0	0	0	0	1.0	0	0	0
Bmag0387	(AG)16	150	3	2	0.32	66.7	0.38	2.25	0.44	0.50	0.035
Bmac0316	(AC) 19	135	3	3	0.44	100	0.37	1.5	0.45	1.0	0.034
Bmag0009	(AG)13	172	2	2	0.81	100	0.22	1.75	0.24	0.50	0.029
Bmag0011	(AG) 25	147	2	1	0.56	50	0.37	0.75	0.46	0.50	0.035
Bmag0135	(AG)10 (GG) 12	152	1	0	0	0	0	1.0	0	0	0
EBmac0603	(CA) 10	149	2	2	0.52	100	0.31	1.63	0.35	0.75	0.023
Total			31.0	22.0	5.33	833.4	3.91	21.48	5.13	12.0	0.37
Average			2.07	1.47	0.36	55.56	0,26	1.34	0.34	0.80	0.02

Note: NA: Number of Alleles, NPB: Number of Polymorphic Bands, PP%: Percentage of Polymorphism (%), PIC: polymorphism Information Content, DI: Diversity Index, EMR: Effective Multiplex Ratio, GR: Discriminating Power, MI: Marker Index, RP: Resolving Power

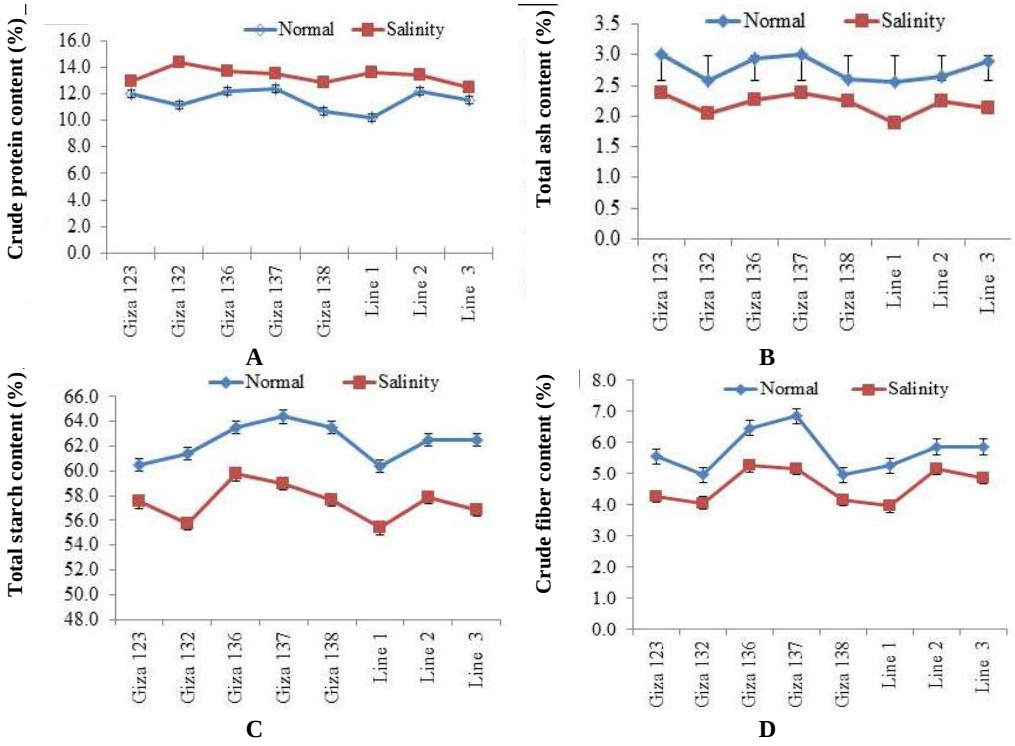


Figure 4. Effect of salinity stress on grain quality traits in the eight Barley genotype

Phenotypic traits under normal conditions

Phenotypic traits under salinity conditions

traits	Cha	Chb	RWC	K+	Na+	SCC	HD	PH	NGS	NT	TKW	GY	CPC	TAC	TSC	CFC
Cha	.711*	.789**	.731*	.883(**)	-.882(**)	-0.47	-.780(*)	.787(*)	.905(**)	.770(*)	0.27	.887(**)	.852(**)	0.00	.787(*)	0.24
Chb	0.651	0.67	.759*	.830(**)	-.625(*)	0.07	-.663(*)	.628(*)	0.54	.634(*)	0.09	0.54	0.55	0.36	0.41	0.07
RWC	0.52	0.561	.750(*)	.872(**)	-.747(*)	-0.37	-.784(*)	.727(*)	0.62	.841(**)	0.05	.694(*)	0.53	-0.03	0.31	0.35
K+	0.66	0.422	0.176	0.51	-.775(*)	-0.31	-.710(*)	.896(**)	.733(*)	.750(*)	0.04	.767(*)	0.61	0.19	0.51	0.40
Na+	-.857(**)	-0.615	-.867(**)	-.833(*)	.912(**)	.707(*)	.923(**)		-.819(**)	-.938(**)	-0.57	-.984(**)	-.822(**)	0.39	-0.59	-0.14
SCC	-0.55	-0.512	-.755(*)	-0.619	.796(*)	0.52	0.60	-0.45	-.647(*)	-.649(*)	-0.45	-.776(*)	-0.57	.759(*)	-0.44	-0.21
HD	-0.58	-0.483	-.714(*)	-.762(*)	0.697	0.457	0.63	-.623(*)	-.702(*)	-.978(**)	-0.45	-.869(**)	-.710(*)	0.35	-0.39	0.03
PH	0.67	0.316	.722(*)	0.687	-0.631	-0.685	-0.527	0.66	.712(*)	.680(*)	0.28	.810(**)	0.50	-0.06	0.53	0.55
NGS	0.773*	0.31	0.439	0.485	-.790(*)	-.795(*)	-.717(*)	0.469	.915(**)	.672(*)	0.21	.870(**)	.728(*)	-0.24	.897(**)	0.45
NT	0.606	0.404	0.407	0.531	-0.674	-0.476	-0.661	0.407	0.385	.810(*)	0.45	.888(**)	.728(*)	-0.36	0.34	0.02
TKW	-0.01	-0.223	0.057	-0.108	-0.445	-0.627	-0.423	0.174	0.072	0.446	0.70	0.53	0.47	-.635(*)	0.21	-0.29
GY	.859(**)	0.594	.748(*)	.862(**)	-.905(**)	-0.658	-.836(**)	.808(*)	.749(*)	.883(**)	0.309	.920(**)	.824(**)	-0.40	.664(*)	0.22
CPC	0.50	0.131	0.019	0.361	-0.251	-0.237	0.041	0.491	0.646	-0.055	-0.027	0.376	0.30	-0.16	.652(*)	-0.15
TAC	0.424	0.317	0.434	0.388	-0.583	-0.436	-0.62	0.246	0.18	0.693	0.415	0.538	.709(*)	-0.13	-0.13	-0.05
TSC	0.14	0.39	0.65	0.286	-0.314	-0.132	-0.596	0.043	0.018	0.615	-0.026	0.196	0.162	-0.123	-0.34	0.42
CFC	0.31	0.193	0.298	0.09	-0.479	-0.463	-0.659	-0.117	0.245	0.624	0.36	0.419	0.608	-0.387	0.042	-0.61

Figure 5. Pearson correlations between the sixteen phenotypic studied traits. Correlations under control conditions are above the diagonal, and correlations under saline conditions are below the diagonal. A heatmap colors these correlations: green indicates significant positive correlations, and red shows significant negative correlations. Within trait correlations between control and saline conditions are positioned on the diagonal alignment (yellow cells). All correlations are significant ($p < 0.01$(**) and $p < 0.05$(*), the abbreviation of traits were shown in Table 3

The Polymorphism Information Content (PIC) value of each SSRs marker ranged from 0.37 (Bmac 0009) to 0.85 (Bmag770), with an average value of 0.36. The outstanding six primer pairs (Bmag 770, Bmac 0209, EBmac 0701, Bmac 0316, Bmag0009, and EBmac 0603) generated clear fragment patterns with high polymorphism (100%). The SSR (Bmag 770) primer generates high marker efficiency indices such as Number of Alleles (NA), Number of Polymorphism Bands (NPB), Percentage of Polymorphism (PP%), polymorphism information content (PIC), effective multiplex ratio (EMR), resolving power (RP), discriminating power (DP) and marker index (MI) values were (4, 4,100 %, 0.85, 2.61, 2.75, 0.86 and 0.087) respectively.

Genetic similarity and cluster analysis

Genetic relationships among eight Barley genotypes based on fifteen SSR primers data were presented in a UPGMA cluster dendrogram (Figure 6). All genotypes are clearly grouped into three groups. The genetic similarity ranged from low genetic similarity GS 0.34 % between (Line 2 and Line 1) to 0.97 % between (Giza 137 and Giza 123) according to the Jaccard similarity index. Most tolerant and moderate genotypes were clustered in group I and II. On the other hand, the sensitive genotypes Giza 132 and Line1 were located in the III group with GS 0.72 % between them. High results of UPGMA cluster analysis were in agreement with field estimation, which indicated that these genotypes were closely related to each other. In general, this is imitated from their response to salt stress performance during the field evolution.

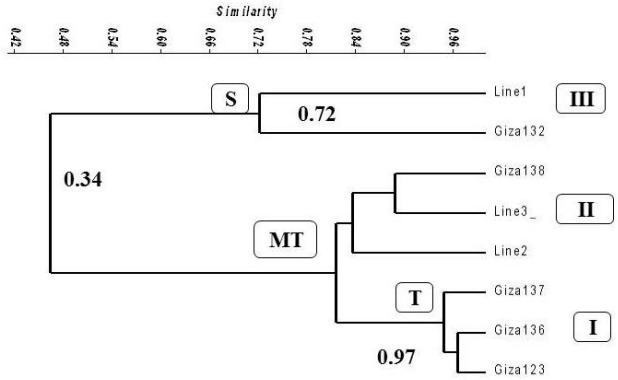


Figure 6. Genetic similarity and UPGMA cluster analysis among the eight Barley genotypes based on SSR markers due to their response to salinity stress. Our results were in agreement with several investigations having studied barley for salinity using SSR markers, such as (El-Akhdar et al. 2016; Mariey et al. 2016; Khatab et al. 2021; Mehta et al. 2021; Mariey et al. 2022a). They used SSR markers to investigate genetic diversity and genetic relationships among Barley genotypes for salt stress conditions, and they reported that the SSRs technique could consider a powerful tool for genetic studies in barley breeding for salinity stress

Relations between genotypes concerning phenotypic and genotypic data under salinity environment (GE)

Bi-plot analysis

Bi-plot was used to study the differences and). PCA-Biplot analysis was presented in a horizontal axis using 16 phenotypic traits and 15 SSR primers to design the relationship trend and categorize barley genotypes under soil salinity stress. The first and two principal components accounted for 97 % (PCA1= 83.5 % + PCA2 =13.5 %) of the total variability, which was graphically displayed in (Figure 7). The eight barley genotypes were divided into three groups. The salt-sensitive genotypes (Line 1 and 132) group was influenced by Na^+ and TSC located in the down left side (negative) of the horizontal axis according to their negative effect correlations with most other traits. Tolerant genotypes (Giza 123, Giza 137, and Giza 136) are more influenced by the molecular primers, and almost of phenotypic traits located on the right side (positive) of the horizontal axis according to their positive effect correlation with them under salinity stress. The modreted salt tolerance genotypes was influenced by HD which locted up left side (negative) of the horizontal axis according to their negative effect correlations with most all traits, but they gave modreted means performace for most of traits.

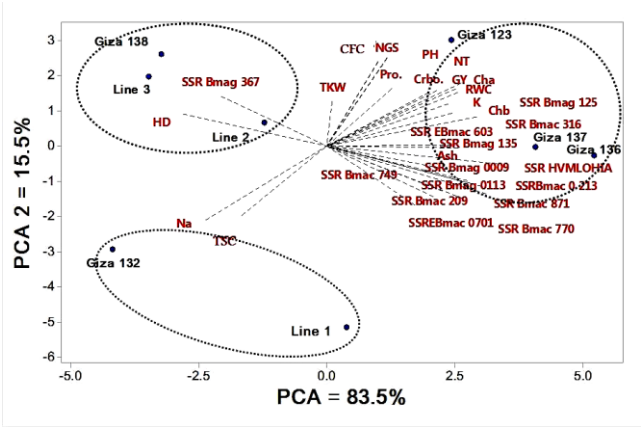


Figure 7. PCA biplot cluster tree illustrates the genetic distance between six-barley based on the analysis of 15 phenotypic traits and genotypic data using 15 SSR primers, all the abbreviation of traits (Tables 3 and 5)

Cluster heatmap

Multivariate heatmap clusters using Euclidean distance and average linkage by R software displayed the interaction between the phenotypic data cluster and molecular data clusters, as shown in (Figure 8). Row dendrograms show that the eight barley genotypes were clustered into two main clusters; the first cluster includes the salinity tolerant genotypes (Giza 123, Giza 136, and Giza 137). The second cluster is divided into two sub-clusters; the first sub consists of sensitive genotypes (Line 1 and Giza 132).

The second sub-cluster includes the moderated salinity divided into sub-sub clusters; moderated salinity (Line 3) and moderated tolerance (Giza 138 and Line 2). It also clearly demonstrates the effects of each field trait on the genotypes and the effects of each initiator molecule on the Egyptian barley genotypes. Column dendrograms show the 16 phenotypic traits and the 15 SSR primers.

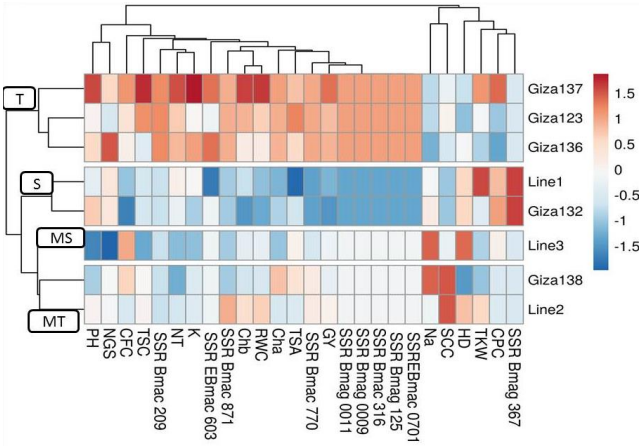


Figure 8. Multivariate heatmap illustrating the genetic diversity of eight Egyptian barley genotypes, based on the 15 SSR primers and 16 phenotypic using the module of a heatmap of ClustVis, all the abbreviation of traits (Table 3)

These results were in good harmony with (Al Lawati et al. 2021; Mariey et al. 2021; Pour-Aboughadareh et al. 2021; Mariey et al. 2022b) They used cluster analysis and principal PCA to classify the barley genotypes into genetic groups as an interaction between environment and genetics data.

The budgets for different barley genotypes under each soil type are shown in Tables 8-9. Although the results show that the total cost of producing different barley genotypes under saline-sodic soil conditions is lower than normal soil conditions, Giza 136 achieved the highest value of Net Return per hectare (NR) ($1780.46 & $927.40) under both normal and saline-sodic soil, respectively.

Table 8. Crop budgets under normal soil

Operation	Total costs ($)							
	Giza 123	**Giza 132**	**Giza 136**	**Giza 137**	**Giza 138**	**Line 1**	**Line 2**	**Line 3**
Grain Yield	2022.40	1408.00	2143.96	2016.00	1897.60	1312.00	1632.00	1525.86
Straw Yield	90.62	95.57	118.64	106.28	107.48	129.02	104.96	112.37
Total Revenue	**2113.020**	**1503.570**	**2262.600**	**2122.280**	**2005.030**	**1441.020**	**1736.960**	**1638.023**
Seeds	39.5	39.5	39.5	39.5	39.5	39.5	39.5	39.5
Land Preparation								
Plowing	25.41	25.41	25.41	25.41	25.41	25.41	25.41	25.41
Leveling	12.71	12.71	12.71	12.71	12.71	12.71	12.71	12.71
Fertilization								
Super Phosphate	7.62	7.62	7.62	7.62	7.62	7.62	7.62	7.62
N-Fertilizer	28.28	28.28	28.28	28.28	28.28	28.28	28.28	28.28
Labor	12.71	12.71	12.71	12.71	12.71	12.71	12.71	12.71
Weeds Control	8.26	8.26	8.26	8.26	8.26	8.26	8.26	8.26
Irrigation	25.41	25.41	25.41	25.41	25.41	25.41	25.41	25.41
Harvest								
Machine	27.45	27.45	27.45	27.45	27.45	27.45	27.45	27.45
Labor	8.89	8.89	8.89	8.89	8.89	8.89	8.89	8.89
Total Variable Costs	175.80	175.80	175.80	175.80	175.80	175.80	175.80	175.80
Fixed Costs								
Rent	285.89	285.89	285.89	285.89	285.89	285.89	285.89	285.89
Total Costs	**482.13**	**482.13**	**482.13**	**482.13**	**482.13**	**482.13**	**482.13**	**482.13**
Net Return	**1630.89**	**1021.43**	**1780.46**	**1640.14**	**1522.90**	**958.88**	**1254.82**	**1156.10**

Table 9. Crop budgets under saline soil

Operation	Total costs ($)							
	Giza 123	**Giza 132**	**Giza 136**	**Giza 137**	**Giza 138**	**Line 1**	**Line 2**	**Line 3**
Grain yield	1136.0	566.4	120.3	1145.6	850.67	608.0	705.43	780.8
Straw yield	85.85	109.08	101.33	96.06	83.21	102.82	55.20	36.91
Total revenue	**1221.85**	**675.84**	**1311.63**	**1241.66**	**933.88**	**710.82**	**760.63**	**817.71**
Seeds	47.40	47.40	47.40	47.40	47.40	47.40	47.40	47.40
Land preparation								
Plowing	25.41	25.41	25.41	25.41	25.41	25.41	25.41	25.41
Leveling	12.71	12.71	12.71	12.71	12.71	12.71	12.71	12.71
Fertilization								
Super phosphate	7.62	7.62	7.62	7.62	7.62	7.62	7.62	7.62
N-fertilizer	17.78	17.78	17.78	17.78	17.78	17.78	17.78	17.78
Labor	12.71	12.71	12.71	12.71	12.71	12.71	12.71	12.71
Weeds control	8.26	8.26	8.26	8.26	8.26	8.26	8.26	8.26
Irrigation	25.41	25.41	25.41	25.41	25.41	25.41	25.41	25.41
Harvest								
Machine	27.45	27.45	27.45	27.45	27.45	27.45	27.45	27.45
Labor	8.89	8.89	8.89	8.89	8.89	8.89	8.89	8.89
Total variable costs	193.64	193.64	193.64	193.64	193.64	193.64	193.64	193.64
Fixed costs								
Rent	190.60	190.60	190.60	190.60	190.60	190.60	190.60	190.60
Total costs	**384.24**	**384.24**	**384.24**	**384.24**	**384.24**	**384.24**	**384.24**	**384.24**
Net return	**837.61**	**291.24**	**927.40**	**857.43**	**549.64**	**326.58**	**376.39**	**433.47**

Note: Price of barley grains (0.32 $ per 1 Kg)

While the lowest value ($958.88&326.58) was obtained with the Barley genotype Line 1 under both normal and saline-sodic soil, respectively, these results are in great harmony with those obtained by (NDSU 2015; Hammami et al. 2020; Mariey et al. 2022a). They study the economic analysis of the effect of salinity stress on the crops, and they reported that in fields with moderately or so highly saline soils, salt-sensitive crops may no longer be viable as profitability is dramatically reduced.

In conclusion, high genetic differences among eight Egyptian barley genotypes under salinity stress conditions based on a comprehensive set of Agro-morph-physio-chemical parameters coupled with SSR markers and economic analyses were investigated in this study. Thus, these genetic differences among Egyptian Barley genotypes could be more efficient in assessing genetic relationships and classifying the eight for their ability to tolerate salinity stress in breeding programs to produce suitable cultivars at normal and salt stress condition. The results provide information about the ability of eight Barley genotypes for salinity tolerance which Giza 123,136 and 137 provided as salt tolerance genotypes, and two new lines (line 2 and line 3) beside Giza 138 provided as moderated salt tolerance genotypes could use as cultivar identification in further barley breeding programs for salt tolerant in Egypt.

A limited study uses agro-physiological and grain chemical composition with DNA markers under salinity stress for barley genotypes. Also, there is not much work for studying the **e**conomic analysis for Barley salt tolerance or do association among phenotypic parameters, genotypic markers, and economic analysis to improve barley genotypes salt tolerance and farmer income together.

As the results from our data, we could use the tolerance genotypes in future research as a good parent to get promising tolerant lines for salinity stress. Furthermore, using them in breeding programs for salinity and exploring the impact of salt tolerance genotypes on the economic value of the crop to increase farmer income in salt-affected areas.

ACKNOWLEDGEMENTS

The authors extend their pride to Barley Research Department, Crop Physiology Research Department, and Seed Technology Research Department, ARC, Giza, Egypt; Water Management Research Institute, Kalubia, Egypt; and Department of Genetics, Faculty of Agriculture, Kafrelsheikh University, Egypt.

REFERENCES

Acosta-Motos JR, Ortuño MF, Bernal-Vicente A, Diaz-Vivancos P, Sanchez-Blanco MJ, Hernandez JA. 2017. Plant responses to salt stress: Adaptive mechanisms. Agronomy 7 (1): 18. DOI: 10.3390/agronomy7010018.

Akhter MS, Noreen S, Mahmood S, Athar H, Ashraf M, Alsahli AA, Ahmad P. 2021. Influence of salinity stress on PSII in Barley (*Hordeum vulgare* L.) genotypes, probeb chlorophyll a fluorescence. J King Saud Univ Sci 101: 239.

Al Lawati A, Nadaf SK, AlSaady NA, Al Hinai SA, Almamari AR, Al Maawali AA. 2021. Genetic diversity of Omani barley (*Hordeum vulgare* L.) germplasm. Open Agric 6: 628. DOI: 10.1515/opag-2021-0038.

Al-Ashkar I, Alderfasi A, Romdhane W, Seleiman MF, El-Said A Rania, Abdullah A. 2020. Morphological and genetic diversity within salt tolerance detection in eighteen wheat genotypes. Plants 9: 287. DOI: 10.3390/plants9030287.

Allel D, Ben-Amar A, Badri M, Abdelly C. 2019. Evaluation of salinity tolerance indices in North African Barley accessions at reproductive stage. Czech J Genet Plant Breed 55: 61-69. DOI: 10.17221/50/2017-CJGPB.

Amiryousefi A, Hyvönen J, Poczai P. 2018. iMEC: Online Marker Efficiency Calculator. Appl Plant Sci 6 (6): e1159. DOI: 10.1002/aps3.1159.

AOAC. 2000. Official Method of Analysis (17th ed.). Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Bakari MS, Abdallah JM, Hella JP. 2018. Adaptation strategies of small-scale agriculture production to climate change impacts in Micheweni, Tanzania. Trop Drylands 3: 60-75. DOI: 10.13057/tropdrylands/t030205.

Bartlett MS. 1937. Properties of sufficiency and statistical tests. J R Stat Soc Ser A 160: 268. DOI: 10.1098/rspa.1937.0109.

Brbaklic L, Trkulja D, MikicS, Mirosavljevic M, Momcilovic V, Dudic B, Procházková, L, Acin V. 2021. Genetic diversity and population structure of Serbian barley (*Hordeum vulgare* L.) collection during a 40-year long breeding period. Agronomy 11: 118. DOI: 10.3390/agronomy11010118.

Chapman HD, Pratt PE. 1978. Methods of Analysis for Soils, Plants and Waters. University of California Division of Agriculture Science, Priced Publication, Oakland.

Cimmyt. 1988. From Agronomic Data to Farmer Recommendations: An Economic work book "DF: 31-33.

Dell'Aversana E, Hessini K, Ferchichi S, Fusco GM, Woodrow P, Ciarmiello LF, Abdelly C, Carillo P. 2021. Salinity duration differently modulates physiological parameters and metabolites profile in roots of two contrasting barley genotypes. Plants 10: 307. DOI: 10.3390/plants10020307.

Doyle JJ, Doyle JL. 1990. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Focus 12: 13-15. DOI: 10.2307/2419362.

Duis M, Hamilton G, Robers JK, PA, Smith F. 1956. Colorimetric method for determination of sugar and related substances. Anal Chem 28: 350-356. DOI: 10.1021/ac60111a017.

Egyptian Environmental Affairs Agency (EEAA). 2016. Egypt State of The Environment Report Ministry. Egypt.

El Sabagh A, Hossain A, Islam MS, Barutcular C, Hussain S, Hasanuzzaman M, Akram T, Mubeen M, Nasim W, Fahad S, Kumar N, Meena RS, Kızılgeçi F, Yıldırım M, Ratnasekera D, Saneoka H. 2019. Drought and salinity stresses in Barley: Consequences and mitigation strategies. Austral J Crop Sci 13: 810-820. DOI: 10.21475/ajcs.19.13.06.p1286.

El-Akhdar A, Abd El-sattar M, Amer K, Kumamaru T. 2016 .Genetic diversity and association analysis among Egyptian barley (*Hordeum vulgare* L.) genotypes with different adaptations to saline conditions analyzed by SSR markers. Austral J Crop Sci 10 (5): 637-645. DOI: 10.21475/ajcs.2016.10.05.p7331.

FAO. 2008. FAO land and Plant Nutrition Management Service. FAO, Rome.

FAOSTAT. 2019. Food and Agriculture Organization of the United Nations Statistical Database. Available online: http://www.fao.org/faostat

Gonzalez L, Gonzalez-Vilar M. 2001. Determination of relative water content. In: Reigosa MJ (eds). Handbook of Plant Ecophysiology Techniques. Kluwer Academic Publishers, Dordrecht.

Grover A, Sharma PC. 2016. Development and use of molecular markers: Past and present. Crit Rev Biotechnol 36: 290-302. DOI: 10.3109/07388551.2014.959891.

Hammami Z, Qureshi AS, Sahli A, Gauffreteau A, Chamekh Z, Ben Azaiez FE, Ayadi S, Trifa Y. 2020. Modeling the effects of irrigation water salinity on growth, yield and water productivity of barley in three contrasted environments. Agronomy 10: 1459. DOI: 10.3390/agronomy10101459.

Hammer Ø, Harper DAT, Ryan PD. 2001. Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4: 1-9.

Hossain MS. 2019. Present scenario of global salt affected soils, its management and importance of salinity research. Intl Res J Biol Sci 1: 1-3.

Isayenkov SV, Maathuis FJ. 2019. Plant salinity stress: Many unanswered questions remain. Front Plant Sci 10: 80. DOI: 10.3389/fpls.2019.00080.

Khatab IA, El-Mouhamady ABA, Mariey SA. 2021. Comprehensive Selectio criteria for high-yielding bread wheat (*Triticum aestivum* L.) hybrids under salinity stress. Egypt J Bot 61 (3): 709-730. DOI: 10.21608/ejbo.2021.65333.1637.

Lin YP, Wu MC, Charng YY. 2016. Identification of a chlorophyll dephytylase involved inchlorophyll turnover in *Arabidopsis*. Plant Cell 28 (12): 2974-2990. DOI: 10.1105/tpc.16.00478.

Mahlooji M, Sharifi RS, Razmjoo J, Sabzalian MR, Sedghi M. 2018. Effect of saltstress on photosynthesis and physiological parameters of three contrasting barley genotypes. Photosynthetica 56 (2): 549-556. DOI: 10.1007/s11099-017-0699-y.

Mariey SA, Ahmed KR, Agwa AME, Farid MA, Serag AM. 2019. Biochemical and molecular genetic markers associated with salt stress tolerance in Egyptian barley cultivars. Egypt J Plant Breed 23: 183-197.

Mariey SA, Farid MA, Khatab IA. 2016. Physiological and molecular characterization of some Egyptian barley (*Hordeum vulgare* L.) cultivars for salt tolerance. Egypt J Genet Cytol 45: 367-382. DOI: 10.21608/ejgc.2016.9588.

Mariey SA, Ghareeb ZE, Shahein AMEA, Meleha AMI, Gomaa HA, Rady AMS, Khatab IA. 2022b. Genetic diversity analysis among some barley genotypes using multivariable and molecular markers under different levels of soil salinity stress. Asian J Plant Soil Sci 7 (1): 219-236.

Mariey SA, Khaffagy AE, Aiad MA, Khatab IA, Ghareeb ZE. 2022a. The influence of the salinity and weed control treatments on some barley cultivars and its associated weeds. J Glob Agric Ecol 13 (2): 26-50.

Mariey SA, Khedr RA, Zayed BA, Elakhdar A. 2017. Genetic variability among Egyptian barley varieties for agro morphological traits under saline soil condition. Egypt J Plant Breed 21: 577-593. DOI: 10.12816/0046352.

Mariey SA, Mohamed AM, Ali HG. 2018. Effect of salinity stress on physiological and biochemical traits of Barley cultivars. Intl J Environ 7: 65-77.

Mariey SA, Mohamed EN, Zeinab GE, Engy AZS. 2021. Genetic diversity of Egyptian barley using agro–physiological traits, grain quality and molecular markers. Curr Sci Intl 10: 58-71.

Mehta G, Muthusamy SK, Singh GP. 2021. Identification and development of novel saltr esponsive candidate gene based SSRs (cg-SSRs) and MIR gene based SSRs (mir-SSRs) in bread wheat (*Triticum aestivum*). Sci Rep 11: 2210. DOI: 10.1038/s41598-021-81698-3.

Metsalu T, Vilo J. 2015. Clust Vis: A web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. Nucleic Acids Res 43: 566. DOI: 10.1093/nar/gkv468.

Moran R. 1982. Formulae for determination of chlorophyll pigments with N,N Dimethylformamid. Plant Physiol 69: 1376-1381. DOI: 10.1104/pp.69.6.1376.

Mwando E, Han Y, Angessa TT, Zhou G, Hill CB, Zhang XG, Li C. 2020. Genome wide association study of salinity tolerance during germination in barley (*Hordeum vulgare* L). Front Plant Sci 11: 1-15. DOI: 10.3389/fpls.2020.00118.

Nadeem M, Tariq MN, Amjad M, Sajjad M, Akram M, Imran M, Kulikov D. 2020. Salinity induced changes in the nutritional quality of bread wheat (*Triticum aestivum* L.) genotypes. Agrivita J Agric Sci 42: 1-12. DOI: 10.17503/agrivita.v42i1.2273.

Naguib MI. 1962. Arapid colorimetric procedure for the estimation of free and conjugated sugars in plant extracts. Zucker 15: 35.

Narimani T, Toorchi M, Tarinejad AR, Mohammadi SA, Mohammadi H. 2020. Physiological and biochemical evaluation of barley (*Hordeum vulgare* L.) under salinity. J Agric Sci Technol 22 (4): 1009-1021.

NDSU. 2015. Soil Health and Land Management. www.ndsu.edu/soilhealth/wp-content/uploads/2015/12/Corn-salinity_6-13-16.pdf. 6-13-16.

Nei M, Li WH. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci 76: 526-5273. DOI: 10.1073/pnas.76.10.5269.

Peter LP, Young VR. 1980. Nutritional Evaluation of Protein Foods. The United Nations University, Japan.

Pour-Aboughadareh A, Sanjani S, Nikkhah-Chamanabad H, Mehrvar mr, Asadi A, Amini A. 2021. Identifcation of salt-tolerant barley genotypes using multiple-traits index and yield performance at the early growth and maturity stages. Bull Natl Res Cent 45: 11. DOI: 10.1186/s42269-021-00576-0.

Rajeswari S, Sood N, Gokhale ST, Subramanian R. 2019. Assessing salt-stress tolerance in barley. Appl Genet 95: 714-722.

Saade S, Maurer A, Shahid M, Oakey H, Schmöckel-Sandra M, Negrão S, Pille K, Tester M. 2016. Yield-related salinity tolerance traits identifed in a Nested Association Mapping (NAM) population of wild barley. Sci Rep 6: 32586. DOI: 10.1038/srep32586.

Saleh-Amal AH, Abd El-Hamid Hoda A, Shaddad MA, E El-badry N. 2017. Assessment the growth and some chemical contents of three barley cultivars under salt stress. J Environ Sci 46: 227-238.

Sallam A, Amro A, EL-Akhdar A, Dawood MFA, Kumamaru T, Stephen Baenziger P. 2018. Genetic diversity and genetic variation in morpho-physiological traits to improve heat tolerance in spring barley. Mol Biol Rep 45: 2441-2453. DOI: 10.1007/s11033-018-4410-6.

Sally T, Peavy Shirley GB, Ruth NV, Hogben D. 1986. OMNITAB 80: An interpretive system for statistical and numerical data analysis. NBS Special Public 701: 1-2. DOI: 10.6028/NBS.SP.701.

Sorkhi F. 2020. Effect of salt stress on physiology and agronomy characteristics of barley cultivars. Agric Eng Intl 22: 69-75.

Varshney RK, Marcel TC, Ramsay L. 2007. A high density Barley microsatellite consensus map with 775 SSR loci. Theor Appl Genet 114: 1091-1103. DOI: 10.1007/s00122-007-0503-7.

Younis A, Ramzan F, Ramzan Y, Zulfiqar F, Ahsan M, Lim KB. 2020. Molecular markers improve abiotic stress tolerance crops: A review. Plants 9: 1374. DOI: 10.3390/plants9101374.

Zeeshan M, Lu M, Sehar S, Holford P, Wu F. 2020. Comparison of biochemical, anatomical, morphological, and physiological responses to salinity stress in wheat and barley genotypes deferring in salinity tolerance. Agronomy 10: 127. DOI: 10.3390/agronomy10010127.

Zhu J, Fan Y, Shabala S, Li C, Lv C, Guo B, Xu R, Zhou M. 2020. Understanding mechanisms of salinity tolerance in barley by proteomic and biochemical analysis of near-isogenic lines. Intl J Mol Sci 21: 1516. DOI: 10.3390/ijms21041516.

ASIAN JOURNAL OF AGRICULTURE
Volume 7, Number 1, June 2023
Pages: 34-46

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g070105

Induction of drought resistance in bell pepper (*Capsicum annuum* var. *grossum*) with osmopriming Polyethylene Glycol (PEG) 4000

ARSITA PRELLIA, SOLICHATUN♥, ARI PITOYO

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia. Tel./fax.: +62-271-663375, ♥email: solichatun@staff.uns.ac.id

Manuscript received: 1 December 2022. Revision accepted: 11 February 2023.

Abstract. *Prellia A, Solichatun, Pitoyo A. 2023. Induction of drought resistance in bell pepper (*Capsicum annuum *var.* grossum*) with osmopriming Polyethylene Glycol (PEG) 4000. Asian J Agric 7: 34-46.* The bell pepper (*C. annuum* var. *grossum*) is an economically valuable chili cultivar. However, in Indonesia, the cultivation of bell peppers is hampered by drought stress. Osmopriming is an alternative method to improve seed quality. That method was used by soaking the seeds in osmoticum solutions, with Polyethylene Glycol (PEG) as the solution. This research examines the effect of osmopriming with PEG 4000 on the germination and growth of bell pepper seedlings under various drought stressors. In 2020, seeds were collected from ripe bell peppers grown by farmers in Surjo Hamlet, Sukabumi Village, Cepogo Sub-district, Boyolali District, Central Java, Indonesia. This study employed a Completely Randomized Design (CRD) with two parameters: PEG concentration and water capacity variations. The concentrations of PEG 4000 utilized were 0, 50, 100, and 150 g/L. The drought stress test is conducted by cultivating primed bell pepper seedlings in planting media with varying water capacities of 100%, 75%, and 50% Space Capacity (SC). Included in the drought group with a moderate stress level is a water capacity of 50% SC. Each treatment was replicated three times. Under drought stress, osmopriming with PEG 4000 accelerated the germination rate and affected seedling growth regarding height, number of leaves, leaf area, wet seedling weight, and shoot-root ratio. However, under drought stress, osmopriming PEG 4000 had no significant effect on the rise in proline and chlorophyll content nor on the decrease in carotenoids and nitrate reductase activity. PEG 150 g/L with 50% SC was ideal for seedling height, leaf number, leaf area, and wet weight, while PEG 100 g/L with 100% SC was optimal for the shoot-root ratio.

Keywords: *Capsicum annuum* var. *grossum*, drought, osmopriming, polyethylene glycol

INTRODUCTION

Among Indonesia's many valuable agricultural products is bell pepper (*Capsicum annuum* L.). Badan Pusat Statistik (2018) reports that after reaching a low point in 2017 with a production of 7.4 thousand tons, bell pepper output increased to 18.15 thousand tons in 2018. Overall output rose by 10.75 kilotons from 2017 to 2018. It demonstrates that bell pepper has a significant demand amongst the general populace of Indonesia. Bell pepper is a valuable commodity because to the fact that it can be utilized both fresh and dry, making it ideal for a wide variety of culinary applications. Warsi (2013) claims that bell peppers are a valuable commodity due to their high vitamin content, antioxidant properties, and versatility in food processing. These numerous advantages bolstered bell pepper's already significant value as a strategic item in the national economy. Bell pepper has enormous potential for growth in Indonesia as there is now a gap between supply and demand, despite its widespread cultivation in subtropical and tropical regions (Arifianto and Kartika 2018).

Several problems, such as plant disease disturbances and environmental stress conditions, continue to limit the success of bell pepper farming in Indonesia. Chili yields are low because of the long dry season, which also contributes to dryness on farmland (Yusniwati et al. 2008). The pepper plant, related to chilies, is very drought- and heat-sensitive and sensitive to light (Aminifard et al. 2010; Tulung and Demmassabu 2011). The rate of growth, maturity and biomass accumulation of farmed plants can all be altered by drought stress.

Seed priming is an alternate method for enhancing seed quality that involves preparing the seeds before planting. Seed priming can prepare seeds to germinate concurrently, grow better, adapt to adverse environmental conditions such as drought, and be more resistant to disease attacks (Lutts et al. 2016). The widespread use of seed priming in horticulture crops is due to its ability to boost crop yields. For example, applying seed priming to bell pepper seeds is anticipated to optimize bell pepper plant growth and induce drought tolerance.

There are various methods for priming seeds, osmopriming being one of them. The seeds are soaked in an osmotic solution, such as sugar, glycerol, sorbitol, mannitol, or Polyethylene Glycol (PEG), for osmopriming (Lutts et al. 2016). It is known that using PEG for seed priming has increased simultaneous germination and induced tolerance to environmental stress. Sorghum seeds (*Sorghum bicolor* L. Moench), soybean seeds (*Glycine max*), wheat seeds (*Triticum aestivum* L.), and rice seeds (*Oryza sativa* L.) have all been successfully induced to develop drought stress resistance (Eivaz 2012; Syaiful et al. 2014; Salah et al. 2015; Zhang et al. 2015).

Plant morphological, anatomical, and physiological responses to drought can be considered (Kasi et al. 2017). Plants respond physiologically to drought stress by

accumulating proline molecules, which operate as osmoregulatory and osmoprotectant chemicals for cell membranes. Compared to optimal environmental conditions, the proline content of plant leaves increased when plants were exposed to drought (Yusniwati et al. 2008). Due to a loss in chlorophyll and an increase in secondary metabolites, drought stress also disturbs the metabolic activity of plants (carotenoids). Drought can also reduce Nitrate Reductase Activity (NRA) by interfering with the absorption of nitrogen fertilizers. Nitrate reductase enzymes contribute to the assimilation of nitrate, which influences plant development and yield. When plants were subjected to drought conditions, nitrate reductase activity decreased relative to optimal environmental circumstances (Fitriana et al. 2011). The nitrate reductase activity (NRA) can be employed as a plant selection measure since it correlates positively with production, dry weight, total nitrogen, and plant yield.

The application of osmopriming to bell pepper seeds to generate drought tolerance has not been performed. Therefore, it has not been as widespread as it has been with other varieties of chilies. The prior studies employed PEG with molecular weights of 6000 and 8000; however, PEG 4000 has not been utilized extensively (Syaiful et al. 2014; Yuanasari et al. 2015; Zhang et al. 2015). Furthermore, the application of PEG 4000 on seeds, followed by their cultivation under varying drought stress circumstances, is also new knowledge uncovered by researchers. Therefore, reviewing growth characteristics is necessary to identify the response of bell pepper plants to drought stress following osmopriming with PEG 4000, given this context.

Given the above context, the problem is stated as follows: (i) Does osmopriming with PEG affect the germination rate of bell pepper (*C. annuum*) seeds? (ii) Does osmopriming with PEG influence bell pepper (C. annuum) seedlings' growth under drought stress? (iii) Does osmopriming with PEG affect the levels of chlorophyll, carotenoids, proline, and Nitrate Reductase Activity (NRA) in bell pepper seedlings (*C. annuum*) under drought stress? (iv) What is the appropriate concentration of osmopriming and PEG treatment for bell pepper (*C. annuum*) seedling growth under drought stress? Therefore, based on the definition of the problem above, the goal of this study is as follows: (i) Determine the effect of osmopriming with PEG 4000 on bell pepper germination rate (*C. annuum*). (ii) Determine the effect of osmopriming with PEG on the growth of bell pepper (*C. annuum*) seedlings under conditions of drought stress. Determine the effect of osmopriming with PEG on the levels of chlorophyll, carotenoids, leaf proline, and activity of the enzyme nitrate reductase in bell pepper seedlings (*C. annuum*) subjected to drought stress. (iii) Determine the appropriate concentration of osmopriming and PEG treatment on the growth of bell pepper (*C. annuum*) seedlings under drought stress.

MATERIALS AND METHODS

Time and place

This research was carried out in October 2020-April 2021 in the greenhouse of the Integrated Laboratory Technical Implementation Unit, the Biology Laboratory, and the Universitas Sebelas Maret (UNS) Integrated Laboratory of the UNS, Surakarta, Central Java, Indonesia.

Materials and tools

Seeds of bell pepper (*C. annuum* var. *grossum*) were employed in this investigation. The seeds came from farmers in Surjo Hamlet, Sukabumi Village, Cepogo Sub-district, Boyolali District, Central Java, Indonesia, in the harvest year 2020. Red pepper seeds were chosen for regeneration because they were mature and ready to be used. The chemicals used included PEG 4000 solution with concentrations of 0, 50, 100, and 150 g/L, 1% tetrazolium, 80% acetone, distilled water, Merck's standard proline compound, 3% sulfosalicylic acid, 0.14 M ninhydrin acid, glacial acetic acid, toluene 99.5%, phosphate buffer with pH 7.2, sodium nitrate (NaNO3) 5 M, N-[1-naphthyl]-ethylenediamine (NED) 0.02%, 1% sulfanilamide acid, hydrochloric acid (HCl) 3 N. The planting medium was a mixture of alluvial soil and manure (2:1).

Germination cups (pot trays), petri dishes, measuring scales, scissors, razor blades, plastic clips, measuring cups, beakers, stir sticks, pH paper, micropipette, tweezers, mortar, pestle, glass funnel, test tube, Whatman filter paper, water bath, stereo microscope, analytical balance, cuvette, UV-VIS spectrophotometer (Perkin Elmer Lambda 25), and vortex mixer are utilized.

Research design

This research is experimental in the laboratory and greenhouse. The research design used was a Completely Randomized Design (CRD) with two factors, namely variations in PEG 4000 osmopriming (0, 50, 100, and 150 g/L) and variations in water availability (100, 75, and 50% space capacity). Based on these two factors, 12 treatment combinations were obtained. Each treatment was carried out in 3 repetitions. The following is the treatment in this study. The combination of treatment of the two factors is presented in Table 1.

Table 1. Combinations of PEG osmopriming variations and space capacity percentages

Space Capacity Variation	PEG 4000 concentration variations			
	P0	**P1**	**P2**	**P3**
C0	P0C0	P1C0	P2C0	P3C0
C1	P0C1	P1C1	P2C1	P3C1
C2	P0C2	P1C2	P2C2	P3C2

Note: P0 = PEG 0 g/L, P1 = PEG 50 g/L, P2 = PEG 100 g/L, P3 = PEG 150 g/L; C0 = 100% Space capacity, C1 = 75% Space capacity, C2 = 50% Space capacity

Procedure

Preparation of bell pepper seeds

Pepper seeds were obtained from farmers in Surjo Hamlet, Sukabumi Village, Cepogo Sub-district, Boyolali District, Central Java, in 2020. The seeds come from ripe bell peppers and are ready for regeneration. Peppers are split, so the seeds are visible and then dried in indirect sunlight. The dried seeds are threshed from their sockets and then selected as reasonable and uniform (color and seed size are uniform).

Osmopriming treatment

PEG 4000 solution was made in four concentration variations, namely 0, 50, 100, and 150 g/L. Bell pepper seeds are soaked in various solutions for 24 hours. Afterward, the seeds are drained in a container with a paper towel and left to air dry for 24 hours (Latifa and Rachmawati 2020). The dried seeds were then stored for the germination test.

Tetrazolium test

A preliminary test is to determine the initial viability of seeds using the tetrazolium test. The preparation of tetrazolium solution begins with a phosphate buffer solution of 9.078 grams of potassium dihydrogen phosphate (KH_2PO_4) dissolved in 1000 mL of distilled water (solution I). Then 11.876 grams of disodium hydrogen phosphate ($Na_2HPO_4.2H_2O$) dissolved in 1000 mL of distilled water (solution II). Next, 400 mL of solution I and 600 mL of solution II (2:3)(v/v) were mixed homogeneously. Finally, ten grams of tetrazolium salt (2,3,5 triphenyl tetrazolium chloride) was added to 1000 mL of buffer solution to make a 1% tetrazolium solution (Zanzibar and Herdiana 2010).

The tetrazolium test was carried out by soaking ten bell pepper seeds in distilled water for one day and one night (24 hours). After 24 hours, the seeds were split into two parts. One part was soaked in a 1% tetrazolium solution of approximately 20 mL for 24 hours at room temperature (± 28°C), and the other was in the dark. Seeds whose embryonic parts and cotyledons are pink to dark red are counted as viable seeds (Varela and Albornoz, 2013). The tetrazolium test was carried out in 3 repetitions. Seeds treated with osmopriming were tested for viability again with the tetrazolium test. The percentage of seed viability is calculated using the following formula (Subantoro and Prabowo 2013).

$$seed\ viability\ = \frac{number\ of\ viable\ seeds}{total\ number\ of\ seeds} \times 100\%$$

Germination test

Seeds that had been treated with osmopriming were germinated on filter paper media. A total of 30 seeds were imbibed in distilled water for 12 hours. The seeds were then germinated in a germination cup that had been lined with filter paper. Each cup contains ten seeds, so there are three replications. The filter paper was moistened with as much as 15 mL of distilled water. Germination incubation was carried out at room temperature for two weeks. Watering with distilled water and the same volume is done every day until the end of germination. Observations were made every day on the time the sprouts appeared, and then the germination percentage and rate were calculated. At the end of germination, the seeds were weighed to determine the wet weight of the sprouts. Seeds are said to have germinated when radicles appear at least 2 mm long (Pramana et al. 2019). The formula for calculating germination percentage and rate is as follows (Sutopo 2002; Lesilolo et al. 2013).

$$germination\ (\%)\ = \frac{number\ of\ germinated\ seeds}{number\ of\ seeds\ germinated} \times 100\%$$

$$germination\ rate\ (days)\ = \frac{N1T1 + N2T2 + \cdots + NxTx}{number\ of\ germinated\ seeds}$$

$$germination\ speed\ index\ = \frac{N1}{T1} + \frac{N2}{T2} + \cdots + \frac{Nx}{Tx}$$

Where:

N = number of seeds that germinate in a particular time unit

T = The time between the test's start and the end at a specific observation interval.

Treatment of drought stress

Drought stress treatment with variations of water stress 100%, 75%, and 50% Space Capacity (SC) lasted for 13 weeks. Space capacity was determined using the gravimetric method. The planting medium is a mixture of ground and manure with a ratio of 2:1.

Seeds treated with the priming variation were sown in the media with 100% watering at Space Capacity (SC) for 14 days beforehand. Seedlings with uniform size were then transferred to polybag media with soil moisture content according to treatment. Seedling uniformity is based on the height and number of seedling leaves. Next, the polybags were placed in greenhouse and watered every two days with the volume according to the planned treatment. After 13 weeks, growth was observed in morphological and physiological parameters.

Determination of space capacity used the appropriate gravimetric method from Swibawa and Oktarino (2010). This method is carried out by weighing the initial weight of the media (Wo) and then pouring the media with water until it is saturated (all the pore space of the media is filled with water) and left for 24 hours or until the water stops dripping from the polybag. After the water has stopped dripping, the media is weighed again to determine the final weight (Wa). The water content for 100% space capacity (Wt) is determined by subtracting the final weight of the media from the initial weight of the media. Space capacities of 75% and 50% are determined by:

Water content to be added = % SC required x Wt

Observation of pepper plant growth

Seedling growth parameters. The growth of pepper seedlings was observed at the end of the drought stress treatment when the plants were 13 weeks old. Growth parameters observed included plant height, fresh weight of seedlings, root crown ratio, leaf area, and the number of leaves.

*Measurement of plant height***.** Plant height is measured from the base of the stem above the soil surface to the apical growing point. (ii) Root canopy ratio is the ratio between the wet weight of the upper part of the plant (leaves, stems) and the lower part of the plant (roots). The formula for measuring the crown root ratio is as follows (Effendi 2008):

$$root\ crown\ ratio\ (gram) = \frac{crown\ wet\ weight}{root\ wet\ weight}$$

Measurement of leaves. Leaves measured in width are leaves that have opened completely. Leaf area measurement uses the gravimetric method and is calculated using the following formula (Irwan and Wicaksono 2017).

$$leaf\ area\ (cm^2) = \frac{leaf\ replica\ paper\ weight}{total\ paper\ weight} \times total\ paper\ area$$

Physiological parameters. The measured physiological parameters were chlorophyll, carotenoids, leaf proline levels, and the enzyme nitrate reductase.

Measurement of leaf proline content. Analysis of total leaf proline content was carried out using Bates et al. (1973). Fresh leaves (2nd to 3rd leaves from shoots) as much as 0.1 gram, cleaned, then mashed and homogenized with 5 mL of sulfosalicylic acid (3% w/v). After centrifuging at 5000xg for 15 minutes using a centrifuge, 2 mL of the supernatant obtained was taken and reacted with 2 mL of 0.14 M ninhydrin acid ($C_9H_6O_4$) solution (1.25-gram ninhydrin composition) and added with 2 mL of Glacial Acetic Acid (GAA) then heated using a water bath at 100°C for 60 minutes.

The test tube containing the supernatant that has been heated is put into a beaker filled with ice cubes for 5 minutes. The sample is mixed with 4 mL of toluene (99.5%) and vortexed for 15-20 seconds until two layers of liquid with different colors are formed. The red toluene-containing proline was taken, and the absorbance value was read with a UV-VIS spectrophotometer at 520 nm. The blank solution is toluene. L-proline (Sigma) dissolved in sulfosalicylic acid (3% w/v) was used as standard.

Measurement of total chlorophyll and carotenoid content of bell pepper leaves. Measurement of leaf chlorophyll and carotenoid content was carried out at the end of the observation. The content of chlorophyll and carotenoids was measured by cleaning fresh leaf samples and weighing 0.1 grams. The sample is mashed with a mortar and pestle. Once smooth, the sample is extracted with 10 mL of 80% acetone, then stirred until the color is released from the tissue. The leaf extract is filtered with filter paper. The filtrate obtained was put into a cuvette of 3 ml. The content of chlorophyll and carotenoids was analyzed using a UV-VIS spectrophotometer at wavelengths of 480 nm, 645 nm, and 663 nm with a blank of 3 mL of 80% acetone (Kurniawan et al. 2010). This method was carried out for each treatment sample. The chlorophyll content was calculated using the equation from Hendry and Grime (1993), as follows (Anggarwulan and Solichatun 2007):

Chlorophyll a (mg/g F.W) = (12.7 x A663) - (2.69 x A645) x 10^{-1}

Chlorophyll b (mg/g F.W) = (22.9 x A645) - (4.68 x A663) x 10^{-1}

Total chlorophyll (mg/g F.W) = (8.02 x A663) + (20.2 x A645) x 10^{-1}

The calculation of carotenoid content using the equation according to Gayathri et al. (2016) is as follows:

Carotenoids (mg/g F.W) = A480 + (0.114 x A 663) (0.638 x A645)

Where:
A663 = Absorbance value at a wavelength of 663 nm
A645 = Absorbance value at a wavelength of 645 nm
A480 = Absorbance value at a wavelength of 480 nm
FW = Fresh weight (wet weight of leaves)

The nitrate-reductase activity measurement. Nitrate reductase activity was measured using the first to third leaf samples from shoots of 0.25 g. The leaves were cut into small pieces and put into a dark bottle containing 5 mL phosphate buffer solution (pH 7.2-7.5) for 24 hours. After 24 hours of incubation, the phosphate buffer solution was replaced with 5 mL of new phosphate buffer solution, and 0.1 mL of 5M $NaNO_3$ was added as a substrate, then incubated for 2 hours (after this, the sample was called an aliquot). Finally, 0.1 mL aliquots were put into a test tube containing the coloring reagent in the form of 0.2 mL of 1% sulfanilamide acid in 3N HCl and 0.2 mL of 0.02% N-Naphtylethylen diamine (NED). Then, wait for the reaction until a pink color change occurs (10-15 minutes) as a sign of nitrate reduction to nitrite by the enzyme nitrate reductase. After a color change, 5 mL of distilled water was added and homogenized. Sample absorbance was measured using a UV-VIS spectrophotometer at 540 nm. The blank solution is a mixture of coloring reagents with a ratio of 1:1. Nitrate reductase activity (NRA) is calculated using the following formula (Latifa and Anggarwulan 2009; Putra et al. 2020).

$$NRA\ (\mu mol\ NO^{2-}/g/h) = \frac{sample\ absorbance}{absorbance\ standard} \times \frac{100}{WW} \times \frac{1}{IT} \times \frac{50}{100}$$

Where:
Absorbance standard = 0.0142
WW = wet weight of the sample (grams)
IT = incubation time (hours)

Data analysis

Quantitative data were analyzed by two-way ANOVA (Analysis of Variance), followed by one-way unstacked

ANOVA, and then tested by Duncan's Multiple Range Test (DMRT) at a 95% confidence level.

RESULTS AND DISCUSSION

Tetrazolium test

Seed viability testing was carried out using the tetrazolium test method on the treated seeds. This method is called the quick test because the seeds tested can be analyzed quickly and do not need to be germinated. Good viability (live seeds) seeds are marked with a red pattern embryo and cotyledons after soaking for 24 hours with 1% tetrazolium solution Figure 1). The tetrazolium test was carried out on seeds that had not been treated (the control) and Polyethylene Glycol (PEG) 4000-treated seeds with concentrations of 50, 100, and 150 g/L, with three replications each. The results of the tetrazolium test yielded a seed viability percentage value of 100% for all control and treated seeds. These results indicate that all seeds are still viable (alive) and can germinate and grow. Therefore, giving PEG 4000 treatment cannot affect seed viability.

The test uses tetrazolium salt (2,3,5 triphenyl tetrazolium chloride) as an indicator to detect the presence of living cells. Every living organism or tissue performs respiration and produces hydroxide gas (H_2) with the help of dehydrogenase enzymes. The dehydrogenase enzyme activity will release H+ ions and react with tetrazolium, which was initially colorless and reduced to a red precipitate called formazan. Therefore, viable seeds absorb tetrazolium and appear red (Copeland and McDonald 2001; Warid and Palupi 2009). The nature of PEG only lowers the osmotic potential of the water in the media and the compound because it has a large molecular weight and does not get absorbed into the tissues or seed embryos, so it does not poison the seeds. Based on this description, applying PEG is safe for seeds and does not affect seed viability.

Germination test

Pepper seeds that had been soaked in PEG 4000 solution according to the treatment were germinated for 14 days. Seeds are germinated when radicles appear at least 2 mm long (Pramana et al. 2019). The seed germination process starts from the process of water absorption by the seed (imbibition). Imbibition occurs in three phases. Phase I begins with rapid water absorption due to the potential difference between the water and the seeds. In phase II, imbibition is slow because there is a potential water balance in the seed with its environment, and seed metabolism is actively taking place. Finally, in phase III, the rate of imbibition increases again because the growth and development of the sprouts are ongoing, which begins with the appearance of the radicle (Yuanasari et al. 2015; Ernita and Mairizki 2019). Table 2 shows the average number of sprouts that appeared each day. It was found that the PEG 100 and 150 g/L treatments on the first day had germinated seeds, while the control treatment sprouts started appearing on the 4th day. The highest number of sprouts in the control treatment appeared on the 7th day. In the PEG 50 and 100 g/L treatments, the highest number of sprouts appeared on 4th day, and in the 150 g/L PEG treatment, the highest number of sprouts appeared on 5th.

In Table 3, it was found that the PEG 4000 control, 50, 100, and 150 g/L treatments produced a 100% germination percentage of bell pepper seeds with a different wet weight for each sprout. The ANOVA results showed that the PEG 4000 treatment significantly affected the fresh weight of each sprout. The 50 g/L PEG treatment produced the heaviest sprouts of 0.0575 g, which was not significantly different from the control and 100 g/L PEG treatments but significantly different from 150 g/L PEG, the lowest germination weight of 0.0414 g. These results could imply that the PEG 50 g/L and 100 g/L treatments increased the wet weight of the sprouts compared to that without the PEG 4000 osmopriming treatment. Therefore, the sprouts' wet weight reflects the sprouts' structure growing normally and optimally.

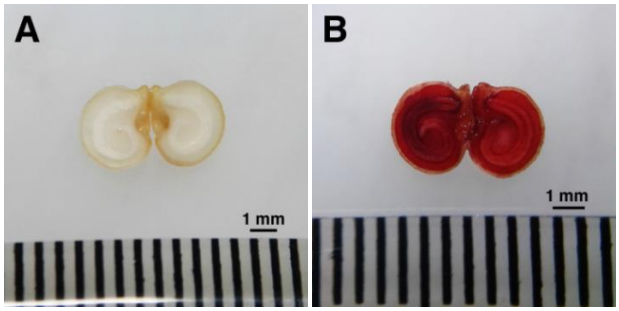


Figure 1. Morphology of cross-sectional peppercorn seeds through a stereo microscope at 1x magnification. Before soaking in 1% tetrazolium solution (A). After soaking in 1% tetrazolium solution (B)

Table 2. Daily germination of pepper seeds

Day	The number of sprouts that appear			
	PEG 0 g/L	PEG 50 g/L	PEG 100 g/L	PEG 150 g/L
1	-	-	0.33	0.67
2	-	0.33	1	0.33
3	-	0.67	1.67	0.33
4	0.33	3.33	2.67	2.33
5	2.67	3	1	3.33
6	2.33	1.33	2.33	1.67
7	3	0.67	0.67	1
8	1	0.67	-	0.33
9	0.33	-	-	-
10	-	-	0.33	-
11	0.33	-	-	-
12	-	-	-	-
13	-	-	-	-
14	-	-	-	-

Table 3. Percentage of germination and average fresh weight of each bell pepper sprout (*C. annuum* var. *grossum*) after osmopriming treatment with PEG 4000

PEG 4000 variation	Germination percentage (%)	Average wet weight of sprouts (g)
0 g/L	100	0.0497^{ab}
50 g/L	100	0.0575^{a}
100 g/L	100	0.0528^{a}
150 g/L	100	0.0414^{b}

Note: Numbers accompanied by the same letter show no significant difference (P>0.05) according to the 5% DMRT test

Germination rate is an important aspect of plant vigor. The germination rate is determined by the number of days it takes for the radicle or plumule to emerge over a certain period (Sutopo 2002). The results of ANOVA showed that the PEG 4000 treatment significantly affected the germination rate and germination rate index of bell pepper seeds. Figure 2A shows that the control treatment produced the highest germination rate of 6.43 days, while the 100 g/L PEG treatment produced the lowest germination rate of 4.50 days. However, it was not significantly different from the 50 g/L and 150 g/L PEG treatments. The germination rate results indicate the seeds' ability to germinate quickly in that time range. In addition, the Germination Speed Index (GSI) value also supports seeds' ability to germinate quickly.

The average germination rate index value in Figure 2B is in line with the germination rate value. The 100 g/L PEG treatment produced the highest germination rate index value of 2.89 and was significantly different from the control treatment, which produced the lowest germination rate index value of 1.63. According to Lesilolo et al. (2013), the higher the germination rate value indicates that, the more days needed for a germination process, the lower the germination rate index value. Ernita and Mairizki (2019) reported that the soaking treatment of 2.5% PEG 6000 for 6 hours significantly affected the vigor index (germination speed) of soybean seeds, with the highest value of 1.16. Based on the results of this study, PEG 4000 treatment as an osmopriming agent at a concentration of 100 g/L increased the ability of seeds to germinate and accelerate germination rates with better vigor.

In general, the pattern of water absorption in seeds treated with osmopriming was not different from those without treatment. The osmopriming treatment only affects the water absorption rate so that it is slowed down and controlled (Varier et al. 2010). Yuanasari et al. (2015) added that Osmo conditioning causes the potential of the seed environment to be lower so that the initial rate of imbibition (phase I) can be slowed down. Then entering phase II, the seed can complete the pre-germination metabolic process optimally before planting so that the seed is ready for the emergence of radicles (prospective roots) (phase III). Based on this description, giving PEG 100 g/L in this study was thought to slow and control the rate of imbibition in phase I so that the seeds could improve and complete their metabolic processes during phase II before entering phase III. When seeds are planted or germinated, they are ready to germinate (phase III). The faster germination rate parameter and the high germination speed index value indicate it.

Growth of pepper seedlings

Seedling height

In general, seedlings treated with drought stress and PEG 4000 produced higher seedling height than the control. The results of ANOVA show that the treatment of variations in water availability and PEG 4000 independently or in combination significantly affects the height of the bell pepper seedlings (Figure 3).

Table 4 shows that water availability (space capacity) significantly affected seedling height growth in the control treatment without PEG 4000 treatment. PEG 4000 treatment at 100% SC water availability caused the average seedling height to increase and decrease, although not significantly. At 75% SC water availability, PEG 50, 100, and 150 g/L treatment decreased the seedlings' average height, which was not too significant. Meanwhile, the availability of water at 50% SC of seeds primed with PEG 4000 increased the average height of the seedlings significantly. However, PEG concentrations of 100 and 150 g/L produced a height that was not significantly different. Based on these results, water availability at 75% SC without PEG treatment was the optimum condition for the seedlings, and did not consider it to be in a stressed environment. It was evident in the 75% SC control, which had a better height than the 100% SC control. The PEG treatment of 150 g/L was the optimum concentration for pepper seedlings because it produced the highest seedling height compared to the control, even in low water availability (50% SC).

According to Rahayu et al. (2005), polyethylene glycol (PEG) can mimic drought stress conditions because this compound lowers the osmotic potential of the solution through the activity of the ethylene oxide subunit matrix, which can bind water molecules with hydrogen bonds. In this study, the PEG 4000 treatment increased the height of bell pepper seedlings when there was water stress. Presumably, the seedlings selected with PEG had been simulated to be in a stressed environment so that when grown on planting media, they were able to adapt under unfavorable conditions (low water availability). In addition, the process of cell division and expansion in the apical meristem was not hampered, resulting in taller pepper seedlings.

Table 4. The average height of bell pepper (*C. annuum* var. *Grossum*) seedlings after treatment with drought stress and PEG 4000

Variation of space capacity	PEG 4000 concentration variations			
	0 g/L	50 g/L	100 g/L	150 g/L
100% SC	13.90^{e}	22.67^{b}	21.23^{bc}	21.00^{bc}
75% SC	21.07^{bc}	18.67^{cd}	17.40^{d}	17.40^{d}
50% SC	10.57^{f}	20.20^{bcd}	28.27^{a}	31.20^{a}

Note: Numbers accompanied by the same letter indicate no significant difference (p>0.05) according to the 5% DMRT test

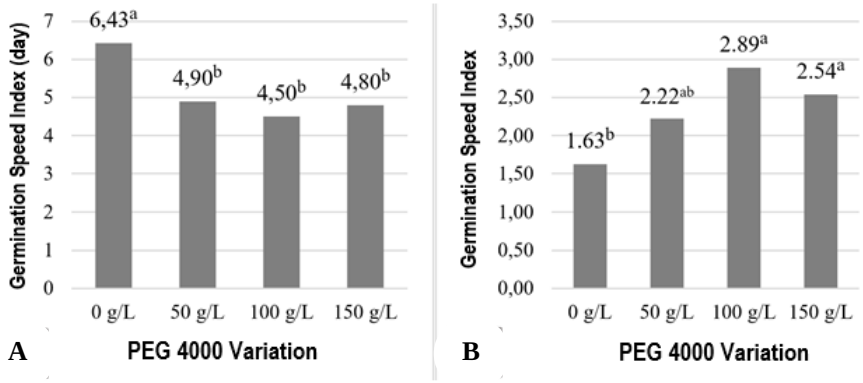


Figure 2. Average germination rate (A) and index seed germination rate (B) after osmopriming with PEG 4000. Notes: Numbers accompanied by the same letter indicate no significant difference (P>0.05) according to the 5% DMRT test

Figure 3. Compilation of photos of pepper seedlings aged 13 weeks after treatment

Drought stress treatment can cause a significant reduction in plant height (Armita et al. 2017). According to Yusniwati et al. (2008), drought stress negatively impacts chili growth. Reducing the provision of water can cause vegetative growth such as plant height, root length, plant dry weight, and generative growth of chili to decrease compared to plants at optimum conditions. Subantoro (2014) added that a lack of water conditions could cause a decrease in cell turgor, affecting cell division and expansion, thereby inhibiting vegetative growth and decreasing physiological processes. Research from Nurjannati (2017) reported that the PEG 6000 treatment with a concentration of 250 g/L with 600 mL of water (equivalent to 100% SC) had an effect on the height of the chili plants with the highest average value (38.17 cm) compared to giving PEG 225 g/L and the control. The combination of 150 g/L PEG treatment with 50% SC water capacity resulted in the highest average seedling height of 31.20 cm. This treatment combination is thought to have the ability to adapt to drought stress so that the reduction in water content does not significantly hinder the growth of seedling height. The PEG 0 g/L combination shows the lowest value with a water capacity of 50% SC, which is 10.57 cm.

Number and area of leaves

The results of ANOVA show that the treatment of variations in water availability and PEG 4000 independently or in combination significantly affected the number of leaves and leaf area of the bell pepper seedlings. Leaf area will decrease if the intensity of drought stress is high. The average number and leaf area of bell pepper seedlings (*C. annuum* var. *grossum*) after drought stress and PEG 4000 treatment are presented in Table 5.

Table 5 shows that the number of leaves at 100% SC water availability with PEG 4000 treatment can increase the number of leaves. However, the number of leaves decreases as the PEG concentration increases, although not significantly different. At 75% SC water availability, PEG 4000 can reduce the number of leaves with values that are not significantly different. The highest average number of leaves was obtained in the 150 g/L PEG treatment with 50% KL water availability, namely 21 leaves, while the lowest average number of leaves was obtained in the 0 g/L PEG treatment with 50% KL water availability, namely eight strands. Generally, pepper seedlings with a water availability of 100%-50% SC primed with PEG 4000 could still produce good leaves. It is suspected that the plants did not experience growth inhibition due to drought stress so that they could grow more leaves. According to Samanhudi et al. (2021), a decrease in the number of leaves is a form of plant adaptation to water shortage conditions. If the number of leaves decreases, then the transpiration rate also decreases.

In the leaf area parameter, treatment without PEG 4000 (the control) can reduce leaf area at 50% SC water availability. In comparison, at 75% SC water availability, there is a significant increase in leaf area. It is suspected that the availability of 75% SC water is the optimal limit for plants to grow, so it does not show a significant difference with the treatment of 100% SC water availability. In general, osmopriming with PEG 4000 increased leaf size. The treatment of 100% and 75% SC water availability did not show a significant difference after administration of PEG 4000 at concentrations of 50, 100, or 150 g/L. In contrast, at 50% SC water availability, it produced significant differences after osmopriming PEG 4000, even though the concentration was 100 g/L and 150 g/L were not significantly different. The PEG concentration of 150 g/L at 50% SC water availability resulted in the highest average leaf area of 14.72 cm^2. The lowest average leaf area was obtained in the PEG treatment with 0 g/L and water availability of 50% SC which was 1.80 cm^2.

One of the plant responses to drought stress is to reduce leaf area. The decrease in leaf area is related to transpiration which will decrease in rate so that the plants do not lose too much water (Hendrati et al. 2016). According to Hidayati et al. (2017), plants that lack water will inhibit the growth and development of young leaves, cell shrinkage occurs, and the leaf aging process is followed by the dropping of old leaves, resulting in a reduction in the photosynthetic area. Based on this study, osmopriming with PEG 4000 can increase the number and expansion of leaves. Therefore, it is suspected that pepper seedlings can adapt to drought stress conditions. Their transpiration process is at a tolerance limit so that excess water loss does not occur and growth can still run normally.

Seedling wet weight

Plants under drought stress generally experience a decrease in plant wet and dry weight. The results of ANOVA show that the treatment of variations in water availability and PEG 4000 independently or in combination significantly affected the fresh weight of each bell pepper seedling. The average wet weight of the seedlings in Table 6 shows that water availability in the 75% SC control treatment had a significantly different average wet weight per seedling with the 100% and 50% SC controls. This result is in line with the average height of the 75% SC control seedlings, which was assumed to be the optimum condition for seedlings without osmopriming treatment.

Table 5. Average number and leaf area of bell pepper seedlings (*C. annuum* var. *grossum*) after drought stress treatment and PEG 4000

PEG 4000 concentration variations	Variation of space capacity (% SC)	Number of leaves	Leaf area
0 g/L	100	10.67^{de}	3.18^{de}
	75	15.00^{bcd}	5.87^{bcd}
	50	8.00^{e}	1.80^{e}
50 g/L	100	16.33^{b}	8.19^{b}
	75	12.33^{bcde}	4.62^{cde}
	50	14.00^{bcd}	5.36^{bcd}
100 g/L	100	15.33^{bc}	7.58^{bc}
	75	14.33^{bcd}	6.45^{bc}
	50	20.67^{a}	13.53^{a}
150 g/L	100	12.00^{bcde}	6.23^{bc}
	75	11.33^{cde}	6.34^{bc}
	50	21.00^{a}	14.72^{a}

Note: Numbers accompanied by the same letter indicate no significant difference (P>0.05) according to the 5% DMRT test

Table 6. Average fresh weight of bell pepper (*C. annuum* var. *Grossum*) seedlings after drought stress treatment and PEG 4000

Space capacity variation	PEG 4000 concentration variations			
	0 g/L	50 g/L	100 g/L	150 g/L
100% SC	1.11^{de}	4.83^{bc}	5.25^{b}	3.65^{bcd}
75% SC	3.90^{bc}	2.52^{cde}	3.70^{bcd}	2.85^{bcde}
50% SC	0.61^{e}	3.27^{bcd}	13.60^{a}	14.10^{a}

Note: Numbers accompanied by the same letter show no significant difference (P>0.05) according to the 5% DMRT test

Table 7. The average shoot-root ratio of bell pepper (*C. annuum* var. *grossum*) seedlings after drought stress treatment and PEG 4000

Space capacity Variation	PEG 4000 concentration variations			
	0 g/L	50 g/L	100 g/L	150 g/L
100% SC	4.88^{bcd}	6.17^{abc}	8.91^{a}	4.70^{cd}
75% SC	5.81^{bcd}	7.89^{ab}	4.66^{cd}	5.67^{bcd}
50% SC	2.90^{d}	6.98^{abc}	7.35^{abc}	5.74^{bcd}

Note: Numbers accompanied by the same letter indicate no significant difference (P>0.05) according to the 5% DMRT test

The PEG 4000 osmopriming treatment increased and decreased the fresh weight of bell pepper seedlings, which were not significantly different at 100%-75% SC water availability conditions. Whereas at 50% SC water availability conditions showed a significant increase in seedling's wet weight after being treated with PEG 4000. Concentration 150 g/L PEG produced the highest average wet weight of each bell pepper seedling at 15.10 grams, while the lowest wet weight of seedlings was found in the PEG 0 g/L treatment with 50% SC water availability, which was 0.61 grams. This study resulted in the size of the seedlings' wet weight in line with the height of seedlings and number of leaves. The higher the seedling height and the number of leaves, the greater the wet weight of the seedling.

Root header ratio

Drought stress can suppress plant growth, shoots, and roots, causing a decrease in total plant dry weight. In addition, drought stress suppresses shoot development to a greater extent than root development. It is due to the efforts of plants to adapt by reducing water loss through leaves so that plants reduce the crown size and maintain root development to meet sufficient water and nutrient needs (Jamilah 2012). The results of ANOVA showed that the treatment of variations in water availability independently had no significant effect on the shoot-root ratio of the bell pepper seedlings. In contrast, the PEG 4000 treatment and the interaction between the two significantly affected the shoot-root ratio of the bell pepper seedlings.

Table 7 shows that the treatment without the addition of PEG 4000 (control) experienced a decrease in the average crown-root ratio at 50% SC water availability, while at 100% and 75% SC water availability, there was an average increase in the crown-root ratio which was not significant. Therefore, the PEG 4000 treatment was generally not significantly different from the average crown-root ratio under drought-stress conditions. Giving PEG 100 g/L at 100% SC water availability produced the highest average crown-root ratio at 8.91. The lowest shoot-root ratio was obtained in the PEG treatment of 0 g/L available water 50% SC which was 2.90. These results are in accordance with the research of Nurjannati (2017), who reported that the treatment of PEG administration by giving the volume of water to chili plants did not significantly affect the wet weight of the shoot-root ratio. The highest shoot-root ratio wet weight was obtained at 0.69 in the PEG treatment 0 g/L (control) with a volume of 600 mL water (equivalent to 100% SC). In comparison, the PEG treatment was 225 g/L and 250 g/L with a volume of 300 mL water (equivalent to 50% SC), equal to 0.65 and 0.59, with results that are not significantly different.

The crown-root ratio value indicates biomass allocation between the crown and roots during growth. According to Effendi (2008), a decrease in the crown-root ratio illustrates that the assimilate partitions in plant growth under drought conditions will be more distributed to form roots. Water stress can change the distribution or partition of assimilate between organs. The growth of the crown will decrease more than the roots because there is a large water deficit in the crown. A decrease in the crown-root ratio indicates that the plant is under drought stress and, in response, increases the volume and root elongation to absorb water. Based on the results of this study, the application of PEG 4000 generally increased the value of the crown-root ratio. It is suspected that plants allocate assimilate more aimed at the crown, so that shoot growth is good. Pepper seedlings treated with PEG 4000 could grow normally even under stress, so it can be said that these seedlings were tolerant to drought stress.

Leaf proline content

The mechanism of plant tolerance to drought stress is to maintain turgor pressure manifested by a decrease in osmotic potential and the ability to accumulate dissolved compounds in the form of sugars and amino acids, especially proline (Novenda and Nugroho 2016). This study shows obtained proline levels from the regression equation as measured by the proline standard curve $y = 0.3194x - 0.032$ with $R^2 = 0.9063$. The results of ANOVA showed that the treatment of drought stress and PEG 4000 independently or the interaction between the two had no significant effect on the average proline of bell pepper seedling leaves.

Table 8 shows the average response of the proline content of bell pepper leaves to the administration of PEG 4000, and water availability produces values that are not significantly different. Proline levels in the treatment without PEG 4000 with water availability of 75% SC increased and decreased at 50% SC. The highest proline content was obtained in the PEG 50 g/L treatment combination with 100% SC water, which was 1.64 M. The lowest proline content was in the 150 g/L PEG treatment with 75% SC water, which was 1.10 M. Moreover, treatment without administration of PEG 4000 at 75% SC water availability increased proline levels, meaning that

under these conditions, the plants were in a stressed environment, so they responded by accumulating proline. However, at 50% SC water availability, the proline levels decreased. It could be because the bell pepper plants in this study grew optimally at a water availability of 75% SC. When faced with a water availability of 50% SC, the plants assumed they were in a stressed condition, so their growth was disturbed (seen from the low plant height and wet weight), and the plants could not develop. Even if they adapt well, then the measured proline levels are low.

Yusniwati et al. (2008) reported that leaf proline content in plants experiencing drought stress was higher than in the optimum environment. One of the mechanisms of plant adaptation to drought stress is by regulating the osmotic potential of cells, namely through increasing leaf proline concentrations. Proline organic compounds can reduce cell osmotic potential without inhibiting enzyme function and not reducing cell turgor.

The treatment of PEG 4000 with a concentration of 50 g/L resulted in a decrease in proline levels along with low water availability (100-50% SC). The addition of PEG 100 g/L at 100%-75% SC water availability increased proline levels, and at 50% SC decreased leaf proline levels. Adding PEG 150 g/L at 100%-75% water availability decreased proline levels, and at 50% SC water availability increased leaf proline levels. According to Juswardi and Tanzerina (2003), giving PEG can lower the water potential, causing reduced cell turgor pressure. To maintain turgor pressure, plants accumulate dissolved compounds, one of which is proline. PEG-selected plants face unfavorable conditions, so they respond by changing their metabolism affecting plant growth and development. As a result, the growth of selected plants is better than non-selected plants. Effendi (2008) added that plants that are tolerant to drought stress would have higher proline levels.

The average proline content of bell pepper leaves with the control treatment and PEG 4000 administration on water availability produced values that were not significantly different. Therefore, it indicated that there was no interaction between the drought stress treatment and the PEG 4000 given on the proline content of the bell pepper seedling leaves. Therefore, based on the results of this study, a PEG concentration of 50 g/L can induce the resistance of bell pepper seedlings to water stress because there is no increase in proline levels.

Total chlorophyll and carotenoid content

Plants that experience drought stress can disrupt their metabolic activity. It is characterized by a decrease in chlorophyll content and an increase in secondary metabolites, one of which is carotenoid (Armita et al. 2017). The results of the ANOVA of this study showed that the treatment of variations in water availability and PEG 4000 independently or the interaction between the two did not significantly affect the average total chlorophyll in the bell pepper seedling leaves. Furthermore, the results of the ANOVA showed that the PEG 4000 treatment independently affected the carotenoid average of bell pepper seedling leaves, while the independent variation of water availability and the interaction between the two treatments did not affect carotenoid averages.

Table 8. Average proline content of bell pepper (*C. annuum* var. *grossum*) seedling leaves after drought stress treatment and PEG 4000

Variation of space capacity	PEG 4000 concentration variations			
	0 g/L	50 g/L	100 g/L	150 g/L
100% SC	1.14^{ab}	1.64^{a}	1.16^{ab}	1.41^{ab}
75% SC	1.46^{ab}	1.19^{ab}	1.58^{ab}	1.10^{b}
50% SC	1.12^{ab}	1.06^{b}	1.22^{ab}	1.46^{ab}

Note: Numbers accompanied by the same letter show no significant difference (P>0.05) according to the 5% DMRT test

Table 9 shows the average total chlorophyll response of bell pepper seedlings at 75% and 50% SC water availability which was lower than normal conditions (100% SC), although not significantly different. Giving the PEG 4000 at 100% SC water availability increased the total chlorophyll content of the leaves. At 75%-50% SC available water and 50 g/L PEG concentration, there was an increase in the total chlorophyll content of the leaves, but the total chlorophyll content decreased at 100 g/L and 150 g/L PEG concentrations. The highest leaf chlorophyll content was obtained in the treatment of 50 g/L PEG concentration and 100% SC water availability at 1.55 mg/g wet weight. The lowest chlorophyll content was obtained in the treatment with a PEG concentration of 150 g/L with a water availability of 75% SC at 1.19 mg/g wet weight. Following Zhang et al. (2015), priming PEG 8000 with a concentration of 20% (w/v) on sorghum seeds (*S. bicolor*) can increase total chlorophyll content (chlorophyll a and b) in conditions of poor soil moisture (dryness) with a content of 1.68 mg/g wet weight compared to seeds that were not primed had a total chlorophyll content of 1.39 mg/g wet weight. Syaiful et al. (2014) reported that seeds primed with PEG 300 g/L produced soybean plants that were tolerant to drought stress (50% SC), with the highest chlorophyll, protein, and dry weight content.

According to Hendriyani and Setiari (2009), the low water content in the growing media can inhibit chlorophyll synthesis in the leaves. In addition, plants will experience an increasing temperature and transpiration, causing the disintegration of chlorophyll and affecting the decrease in the rate of photosynthesis. Finally, it will result in decreased synthesis of chlorophyll. In general, applying PEG 4000 to different water availability had no significant effect on the average total chlorophyll of bell pepper seedling leaves. These results suggest that applying PEG 4000 did not make bell pepper seedlings under drought stress conditions (plants already can adapt), so the bell pepper seedlings responded by causing no significant decrease in total chlorophyll content.

Carotenoids are one of photosynthetic pigments that plants need in small amounts. Plants experiencing drought stress have a decrease in chlorophyll content. As a result, more carotenoids are needed to continue the photosynthesis process (Armita et al. 2017). The results of the ANOVA in this study showed that the treatment of variations in water availability and PEG 4000 independently or the interaction between the two did not significantly affect the carotenoid average of bell pepper seedling leaves.

Table 9 shows that variations in water availability had no significant effect on carotenoid content, while giving PEG 4000 concentrations of 100 g/L and 150 g/L gave significant results on carotenoid content compared to the control and PEG 50 g/L. The highest average carotenoid content in bell pepper seedling leaves was obtained in the 50 g/L PEG treatment with 50% SC water availability of 1.09 mg/g wet weight, while the lowest carotenoid content was obtained in the 100 g/L PEG treatment with 75% water availability and 50% SC which is equal to 0.60 mg/g wet weight. Based on the results of this study, PEG 4000 was thought not to make pepper seedlings under drought stress conditions, so the seedlings responded with no significant increase in carotenoid levels.

Nitrate reductase enzyme activity

Nitrate reductase is an enzyme that reduces nitrate ions to nitrite ions. Putra et al. (2020) reported that increasing drought stress would reduce the activity of the nitrate reductase enzyme due to an increase in the water potential gradient between the environment and plant tissues. The results of the ANOVA in this study showed that the PEG 4000 variation treatment independently affected the average nitrate reductase activity of the bell pepper seedlings. In contrast, the independent variation of water availability and the interaction between the two treatments had no significant effect on the average of the bell pepper-seedling nitrate reductase activity.

Table 9. Average total chlorophyll and carotenoid content of bell pepper (*C. annuum* var. *grossum*) seedling leaves after drought stress treatment and PEG 4000

PEG 4000 concentration variations	Variation of space capacity (% SC)	Total chlorophyll (mg/g wet weight)	Carotenoids (mg/g wet weight)
0 g/L	100	1.39^{ab}	1.04^{a}
	75	1.29^{ab}	0.97^{a}
	50	1.34^{ab}	0.99^{a}
50 g/L	100	1.55^{a}	1.05^{a}
	75	1.41^{ab}	1.05^{a}
	50	1.51^{ab}	1.09^{a}
100 g/L	100	1.36^{ab}	0.67^{bc}
	75	1.22^{ab}	0.60^{b}
	50	1.27^{ab}	0.60^{b}
150 g/L	100	1.47^{ab}	0.89^{ab}
	75	1.19^{b}	0.68^{bc}
	50	1.37^{ab}	0.71^{bc}

Note: Numbers accompanied by the same letter indicate no significant difference (P>0.05) according to the 5% DMRT test

Table 10. Average levels of the enzyme nitrate reductase (µmol NO^{2-}/g/hour) of bell pepper (*C. annuum* var. *grossum*) seedling leaves after drought stress treatment and PEG 4000

Variation of space capacity	PEG 4000 concentration variations			
	0 g/L	50 g/L	100 g/L	150 g/L
100% SC	148.27 ^{abc}	131.44 ^{bcde}	133.28 ^{bcde}	103.90 ^{f}
75% SC	156.79 ^{a}	128.26 ^{bcde}	113.87 ^{def}	100.60 ^{f}
50% SC	149.87 ^{ab}	134.38 ^{bcd}	127.30 ^{cde}	112.24 ^{ef}

Note: Numbers accompanied by the same letter show no significant difference (P>0.05) according to the 5% DMRT test

Table 10 shows the average response of the activity of the nitrate reductase (NRA) enzyme in bell pepper seedling leaves to the availability of water without PEG 4000 treatment; there were increases and decreases with values that were not significantly different. At the availability of water 100%, 75%, and 50% SC, PEG 4000 administration causes a decrease in the average activity of the enzyme nitrate reductase. The highest average NRA was obtained in the 0 g/L PEG treatment with 75% SC water availability, which was 156.79 µmol NO^{2-}/g/hour, while the lowest NRA average was obtained in the 150 g/L PEG treatment with 75% water availability. % SC is 100.60 µmol NO^{2-}/g/hour. The treatment of giving PEG 4000 at concentrations of 50 g/L and 150 g/L with 50% SC water availability showed an increased NRA value. A PEG concentration of 50 g/L produced a higher NRA value than a PEG concentration of 150 g/L; this was suspected to be an optimum PEG administration, and 50 g/L can still support nitrate reductase activity. The treatment of giving PEG 100 g/L with 50% SC water availability showed a decrease in NRA values.

An increase in the NRA value is suspected that plants can defend themselves against drought stress. A high nitrate reductase value indicates that water in the environment is available, which acts as a provider of protons and electrons for its activities. Water available in the soil can facilitate the transport of nitrogen from the soil into the plant. Fitriana et al. (2011) reported that soil with 100% - 90% SC water availability resulted in higher nitrate reductase activity than that with 70%, 50%, 30%, and 10% SC water availability in *Burangrang* cultivar soybean. These results were suspected because a lack of water can cause stomata to close, thereby cutting off the supply of CO_2 to the mesophyll cells and reducing the photosynthesis rate. Low photosynthetic efficiency affects the amount of NADPH formed in the light reaction (Salisbury and Ross 1992). Suppose there is not an adequate supply of NADPH2 in the cytosol. In that case, there will be a decrease in the nitrate reductase enzyme activity because $NADPH_2$ plays an important role as a proton and electron donor, which can stimulate the movement of electrons in the cytosol (Fitriana et al. 2011).

Based on this research, it can be said that giving PEG 4000 to drought stress can reduce nitrate reductase activity, which is indicated by a decrease in its levels. For example, pepper seedlings treated with 150 g/L PEG priming had low nitrate reductase activity. However, the seedlings showed good growth in seedling height, leaf number and area, and the seedling's wet weight.

The conclusions of this study are (i) Osmopriming with PEG 4000 can accelerate the germination rate of bell pepper (*C. annuum* var. *grossum*) seeds. Giving PEG 100 g/L resulted in the fastest germination rate of 4.50 days with a better vigor value. (ii) Osmopriming with PEG 4000 affected the growth of bell pepper (*C. annuum* var. *grossum*) seedlings, namely increasing seedling height, number of leaves, leaf area, wet weight of seedlings, and crown-root ratio values under drought stress conditions. (iii) Osmopriming with PEG 4000 had no significant effect on increasing total proline and chlorophyll levels but

affected reducing carotenoid levels and activity of the enzyme nitrate reductase of bell pepper seedlings (*C. annuum* var. *grossum*) under drought stress conditions. The highest levels of proline and chlorophyll were obtained in the treatment combination of PEG 50 g/L with 100% SC water availability, while the highest levels of carotenoids were obtained in the treatment combination of PEG 50 g/L with 50% SC water availability. The highest nitrate reductase activity was obtained with the treatment combination of PEG 0 g/L with 75% SC water availability. (iv) The combination of PEG 4000 osmopriming treatment under drought stress conditions optimal for growth of seedling height, number of leaves, leaf area, and wet weight of pepper seedlings was PEG 150 g/L with 50% SC water availability. In contrast, the optimal combination on the canopy ratio parameter- root is PEG 100 g/L with 100% SC water availability.

REFERENCES

Aminifard MH, Aroiee H, Karimpour S, Nemati H. 2010. Growth and yield characteristics of bell pepper pepper (*Capsicum annum* L.) in response to plant density. Asian J Plant Sci 9: 276-280. DOI: 10.3923/ajps.2010.276.280.

Anggarwulan E, Solichatun. 2007. Kajian klorofil dan karotenoid *Plantago major* L. dan *Phaseolus vulgaris* L. sebagai bioindikator kualitas udara. Biodiversitas 8 (4): 279-282. DOI: 10.13057/biodiv/d080407. [Indonesian]

Arifianto M, Kartika JG. 2018. Proses pemanenan bell pepper (*Capsicum annum* var. tribeli) di Greenhouse, De Lier, Belanda Selatan, Belanda. Bul Agrohorti 6 (3): 372-381. DOI: 10.29244/agrob.v6i3.21104. [Indonesian]

Armita D, Arumningtyas EL, Mastuti R. 2017. Tolerance level of three genotypes of cayenne pepper (*Capsicum frutescens* L.) toward drought stress of vegetative phase based on morphological and physiological responses. Intl J Chem Tech Res 10 (2): 183-192.

Badan Pusat Statistik. 2018. Statistik Tanaman Sayuran dan Buah-Buahan Semusim Indonesia. Indonesia: BPS-Statistics Indonesia. [Indonesian]

Bates IS, Waldren RP, Tare ID. 1973. Rapid determination of free proline for water stress studies. Plant Soil 39: 205-207. DOI: 10.1007/BF00018060.

Copeland LO, McDonald MB. 2001. Principles of Seed Science and Technology 4th Edition. Kluwer Academic Publishers, London. DOI: 10.1007/978-1-4615-1619-4.

Effendi Y. 2008. Kajian Resistensi Beberapa Varietas Padi Gogo (*Oryza sativa* L.) terhadap Cekaman Kekeringan. [Tesis]. Program Pascasarjana, Program Studi Agronomi, Universitas Sebelas Maret Surakarta. [Indonesian]

Eivaz A. 2012. Induction of drought tolerance with seed priming in wheat cultivars (*Triticum aestivum* L.). Acta Agriculturae Slovenica 99: 21-29. DOI: 10.2478/v10014-012-0003-6.

Ernita E, Mairizki F. 2019. Penggunaan polietilen glikol sebagai teknik invigorasi untuk memperbaiki viabilitas, vigor, dan produksi benih kedelai. Jurnal Ilmiah Pertanian 16 (1): 8-18. DOI: 10.31849/jip.v16i1.2140. [Indonesian]

Fitriana J, Pukan KK, Herlina L. 2011. Aktivitas enzim nitrat reduktase kedelai kultivar burangrang akibat variasi kadar air tanah pada awal pengisian polong. Biosaintifika J Biol Biol Educ 1 (1): 1-8.

Gayathri N, Gopalakrishnan M, Sekar T. 2016. Phytochemical screening and antimicrobial activity of *Capsicum chinense* Jacq. Intl J Adv Pharmac 5: 12-20.

Hendrati RL, Rachmawati D, Pamuji AC. 2016. Respon kekeringan terhadap pertumbuhan, kadar prolin dan anatomi akar *Acacia auriculiformis* Cunn., *Tectona grandis* L., *Alstonia spectabilis* Br., dan *Cedrela odorata* L. Jurnal Penelitian Kehutanan Wallacea 5 (2): 123-133. DOI: 10.18330/jwallacea.2016.vol5iss2pp123-133. [Indonesian]

Hendriyani IS, Setiari N. 2009. Kandungan klorofil dan pertumbuhan kacang panjang (*Vigna sinensis*) pada tingkat penyediaan air yang berbeda. Jurnal Sains dan matematika 17 (3): 145-150. [Indonesian]

Hidayati N, Hendrati RL, Triani A, Sudjino. 2017. Pengaruh kekeringan terhadap pertumbuhan dan perkembangan tanaman nyamplung (*Callophylum inophyllum* L.) dan johar (*Cassia florida* Vahl.) dari provenan yang berbeda. Jurnal Pemuliaan Tanaman Hutan 11 (2): 99-111. DOI: 10.20886/jpth.2017.11.2.99-111. [Indonesian]

Irwan AW, Wicaksono FY. 2017. Perbandingan pengukuran luas daun kedelai dengan metode gravimetri, regresi dan scanner. Jurnal Kultivasi 16 (3): 425-429. DOI: 10.24198/kultivasi.v16i3.14448. [Indonesian]

Jamilah N. 2012. Pengujian Karakter Morfologi untuk Evaluasi Ketahanan Kekeringan Beberapa Varietas Kedelai (*Glycine max* (L). Merril). [Skripsi]. Jurusan Biologi, Fakultas Sains dan Teknologi, Universitas Islam Negeri Maulana Malik Ibrahim Malang, Malang. [Indonesian]

Juswardi, Tanzerina N. 2003. Akumulasi prolin dan indeks toleransi kalus padi yang diseleksi Polietilen Glikol (PEG) secara in vitro sebagai marka toleransi kekeringan. Jurnal Penelitian Sains 14: 92-100. DOI: 10.56064/jps.v0i14.294. [Indonesian]

Kasi PD, Cambaba S, Illing I. 2017. Pemanfaatan mulsa serbuk gergaji untuk mengatasi pengaruh cekaman kekeringan pada bibit tanaman cabai (*Capsicum annuum* L.). Jurnal Dinamika 8 (1): 30-40. [Indonesian]

Kurniawan M, Izzati M, Nurchayati Y. 2010. Chlorophyll, carotenoid and vitamin C in some species of aquatic plants. Bul Anat Physiol 18: 28-40.

Latifa A, Rachmawati D. 2020. Pengaruh osmopriming benih terhadap pertumbuhan dan morfofisiologi tanaman kangkung darat (*Ipomoea reptans* Poir) pada cekaman kekeringan. Jurnal Agronomi Indonesia 48 (2): 165-172. DOI: 10.24831/jai.v48i2.31448. [Indonesian]

Latifa IC, Anggarwulan E. 2009. Nitrogen content, nitrate reductase activity, and biomass of kimpul (*Xanthosoma sagittifolium*) on shade and nitrogen fertilizer variation. Nusantara Biosci 1 (2): 65-71. DOI: 10.13057/nusbiosci/n010203.

Lesilolo MK, Riry J, Matatula EA. 2013. Pengujian viabilitas dan vigor benih beberapa jenis tanaman yang beredar di pasaran Kota Ambon. Agrologia 2 (1): 1-9. DOI: 10.30598/a.v2i1.272. [Indonesian]

Lutts S, Benincasa P, Wojtyla L, Kubala SS, Pace R, Lechowska K, Quinet M, Garnczarska M. 2016. Seed Priming: New comprehensive approaches for an old empirical technique. In: Araujo S and Alma B (Ed) New Challenges in Seed Biology, Basic and Translational Research Driving Seed Technology. https://www.intechopen.com/books/new-challenges-in-seed-biology-basic-and-translational-research-driving-seed-technology/seed-priming-new-comprehensive-approaches-for-an-old-empirical-technique [7 Juli 2020]. DOI: 10.5772/64420.

Novenda IL, Nugroho SA. 2016. Analisis kandungan prolin tanaman kangkung (*Ipomoea reptana* Poir), bayam (*Amaranthus spinosus*) dan ketimun (*Cucumis sativus* L.). Pancaran 5 (4): 223-234. [Indonesian]

Nurjannati K. 2017. Efek Perlakuan Priming terhadap Performa Tanaman Cabai (*Capsicum annuum* L.) pada kondisi stres air. [Skripsi]. Program Studi Biologi, Jurusan Pendidikan Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Negeri Yogyakarta, Yogyakarta. [Indonesian]

Pramana SA, Pujiasmanto B, Sakya AT. 2019. Perbandingan uji tetrazolium dan radicle emergence dalam menduga viabilitas benih kopi arabika (*Coffea arabica* L.). Jurnal Littri 25 (1): 1-10. DOI: 10.21082/littri.v25n1.2019.1-10. [Indonesian]

Putra VP, Solichatun, Sugiyarto, Sutarno. 2020. Nitrate reductase activity of black rice (*Oryza sativa* L.) cempo ireng cultivar strain 13 and 46 as the result og plant breeding using ^{60}Co gamma ray on drought stres variaton. J Phys: Conf Ser 1436 (2020): 1-8. DOI: 10.1088/1742-6596/1436/1/012114.

Rahayu ES, Edi G, Satriyas I, Sudarsono. 2005. Polyethilene Glycol (PEG) dalam media in vitro menyebabkan kondisi cekaman yang menghambat tunas kacang tanah (*Arachis hypogaea* L.). Berkala Penelitian Hayati 11 (2005): 39-48. DOI: 10.23869/bphjbr.11.1.20057. [Indonesian]

Salah S, Yajing G, Dongdong C, Jie L, AAmir N, Qijuan H, Weimin H, Mingyu N, Jin H. 2015. Seed priming with polyethylene glycol regulating the physiological and molecular mechanism in rice (*Oryza sativa* L.) under nano-ZnO stress. Sci Rep 5 (14278): 1-14. DOI: 10.1038/srep14278.

Salisbury FB, Ross CW. 1992. Fisiologi Tumbuhan Jilid 2. Terjemahan Diah R. Lukman & Sumaryono: 1995. Edisi Keempat. ITB-Press, Bandung. [Indonesian]

Samanhudi, Rahayu M, Sakya AT, Susanti YD. 2021. Seleksi ketahanan bebrapa varietas sorgum manis (*Sorghum bicolor* L.) pada berbagai konsentrasi salinitas. Jurnal Pertanian Presisi 5 (1): 40-56. DOI: 10.35760/jpp.2021.v5i1.3740. [Indonesian]

Subantoro R, Prabowo R. 2013. Pengkajian viabilitas benih dengan tetrazolium test pada jagung dan kedelai. Mediagro 9 (2): 1-8. [Indonesian]

Subantoro R. 2014. Pengaruh cekaman kekeringan terhadap respon fisiologis perkecambahan benih kacang tanah (*Arachis hypogaea* L.). Mediagro 10 (2): 32-44. [Indonesian]

Sutopo L. 2002. Teknologi Benih (edisi revisi). Fakultas Pertanian Universitas Brawijaya. PT. Raja Grafindo Persada, Jakarta. [Indonesian]

Swibawa IG, Oktarino H. 2010. Pengaruh kadar air tanah terkontrol terhadap kelimpahan nematoda parasit tumbuhan. Prosiding Seminar Nasional Sains dan Teknologi 3: 213-219. [Indonesian]

Syaiful SA, Dungga NE, Riadi M, Ridwan I. 2014. Seed priming with PEG 8000 for improving drought stress tolerance of soybean (*Glycine max*). Intl J Agric Syst 2 (1): 19-26.

Tulung SMT, Demmassabu S. 2011. Pertumbuhan dan hasil bell pepper (*Capsicum annuum* var. *grossum*) pada beberapa jenis naungan. Eugenia 17 (2): 156-162. DOI: 10.35791/eug.17.2.2011.3538. [Indonesian]

Varela RO, Albornoz PL. 2013. Morpho-anatomy, imbibition, viability and germination of the seed of *Anadenanthera colubrina* var. *cebil* (Fabaceae). Intl J Trop Biol 61 (3): 1109-1118. DOI: 10.15517/rbt.v61i3.11907.

Varier A, Vari AK, Dadlani M. 2010. The subcellular basis of seed priming. Curr Sci 99 (4): 450-456. DOI: 10.12928/pharmaciana.v3i1.415.

Warid, Palupi ER. 2009. Korelasi Metode Pengecambahan In Vitro dan Pewarnaan dalam Pengujian Viabilitas Polen. Makalah Seminar. Departemen Agronomi dan Hortikultura, IPB. [Indonesian]

Warsi AG. 2013. Aktivitas Antioksidan ekstrak metanol buah bell pepper hijau (*Capsicum annuum* L.). Jurnal Ilmiah Kefarmasian 3 (1): 9-19. [Indonesian]

Yuanasari BS, Kendarini N, Saptadi D. 2015. Peningkatan viabilitas benih kedelai hitam (*Glycine max* L. Merr) melalui invigorasi osmoconditioning. Jurnal Produksi Tanaman 3 (6): 518-527. [Indonesian]

Yusniwati, Sudarsono, Aswidinnoor H, Hendrastuti S, Santoso D. 2008. Pengaruh cekaman kekeringan terhadap pertumbuhan, hasil dan kandungan prolin daun cabai. Jurnal Agrista 12 (1): 19-27. [Indonesian]

Zanzibar M, Herdiana N. 2010. Akurasi metode uji cepat dalam menduga mutu fisiologis benih damar. Jurnal Penelitian Hutan 7 (4): 181-189. DOI: 10.20886/jpht.2010.7.4.181-189. [Indonesian]

Zhang F, Yu J, Johnston CR, Wang Y, Zhu K, Lu F, Zhang Z, Zou J. 2015. Seed priming with polyethylene glycol induces physiological changes in sorghum (*Sorghum bicolor* L. Moench) seedlings under suboptimal soil moisture environments. PLoS ONE 10 (10): 1-15. DOI: 10.1371/journal.pone.0140620.

ASIAN JOURNAL OF AGRICULTURE
Volume 7, Number 1, June 2023
Pages: 47-51

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g070106

Evaluation of several fungicides on mycelial growth and conidial germination of *Alternaria* species causing leaf spots in sunflowers under in vitro conditions

JUNISAFITRI MULJOWATI♥, A.R. HIKAM
Department of Microbiology, Faculty of Biology, Universitas Jenderal Soedirman. Jl. Dr. Suparno No. 62, South Purwokerto, Banyumas 53122, Central Java, Indonesia. Tel./Fax.: +62-281-638794, ♥email: juni.muljowati@unsoed.ac.id

Manuscript received: 4 April 2022. Revision accepted: 14 February 2023.

Abstract. *Muljowati J, Hikam AR. 2023. Evaluation of several fungicides on mycelial growth and conidial germination of* Alternaria *species causing leaf spots in sunflowers under in vitro conditions. Asian J Agric 7: 47-51. Alternaria* leaf spot caused by *Alternaria* species is the most destructive disease of sunflowers. Fungicides, such as mancozeb, carbendazim, benomyl, propiconazole, and iprodione, are commonly used to control diseases. However, the continuous use of synthetic fungicides can cause pathogen resistance to these fungicides. Therefore, the aim of this study was to conduct an in vitro test on the effect of fungicides, such as benomyl, carbendazim, mancozeb, iprodione, and propiconazole, on the mycelial growth and germination of conidia of *Alternaria* species causing leaf spot on sunflower. The experiment was performed in a completely randomized design with factorial patterns. The first factor was the type of fungicide, namely benomyl, mancozeb, iprodione, carbendazim, and propiconazole. The second factor was the concentration level of the (0%, 25%, 50%, 75%, 100%, and 125%) recommended dose. Data were Analyzed of Variance (ANOVA) using SPSS 18 version. The results showed that *Alternaria* species were resistant to carbendazim (32.98%) and benomyl (40.32%). It also shows an intermediate level of resistance to mancozeb (62.59%), iprodione (65.38%), and sensitivity to propiconazole (78.38%). Based on the research results, the authors suggest sunflower farmers use propiconazole to control *Alternaria* species. However, such fungicides may trigger the use of fungicides with higher doses than the recommended dose. That led to the emergence of Alternaria species resistant to the fungicides benomyl, carbendazim, mancozeb, and iprodione.

Keywords: *Alternaria*, fungicide effectivity, leaf spot, sensitivity, sunflower

INTRODUCTION

Nowadays, sunflowers have many benefits, including medicine, cosmetics, textile dyes, and food (Purwati and Herwati 2016; Adeleke and Babalola 2020). However, the limiting factor in sunflower cultivation is foliar diseases such as leaf spots caused by *Alternaria* species (Zhang et al. 2021). Symptoms of the disease are dark brown, oval to circular spots with pale margins and a yellow halo. Spots are found on leaves, stems, petioles, sepals, and petals. In severe infections, lesions become irregular by coalescing, leading to blight, defoliation, and plant death. The disease is severe in wet weather and under moist conditions (Bianchini and Bullerman 2014).

Alternaria leaf spot threatens sunflowers production causing yield losses in all production areas that may reach 60-80% (Viriyasuthee et al. 2019). The high destructive potential of leaf spots makes fungicide control an option and requires repeated application of fungicides to prevent yield loss (Aalum et al. 2016). However, the continuous of synthetic fungicides use or not using recommended doses can lead to pathogen resistance to these fungicides (Ons et al. 2020). Benomyl and carbendazim belong to the benzimidazole group of fungicides with a heterocyclic nitrogen component. The benzimidazole's effect on the plant could inhibit the mitochondrial fumarate reductase enzyme, reduces glucose transport, and eliminates oxidative phosphorylation (Allen and Gottlieb 1970). Benomyl attaches to the microtubule, disrupting processes in the cell, such as cell division and intracellular transport (Rouabhi 2010). Furthermore, benomyl inhibits the growth of *Cercospora beticola* (Utlwang et al. 2017). Carbendazim controls pathogens that cause leaf blight in sunflowers (Devi et al. 2015) and *Alternariaster helianthi* (Jena et al. 2020).

Mancozeb is a dithiocarbamate fungicide that acts on the plant surface where it is applied. This fungicide cannot inhibit the development of fungi in plant tissues but is capable of multi-site inhibitors, which can inhibit the growth of fungi through several places in fungal cells (Rouabhi 2010). Furthermore, mancozeb can hinder the development of several phytopathogenic fungi, including *Choanephora cucurbitarum* (Chandrakala et al. 2018) and *A. helianthi* (Jena et al. 2020).

Iprodione is a dicarboximide fungicide widely used worldwide in agriculture (Bitar et al. 2019). It belongs to the dicarboximide group, which inhibits steroidogenesis (Blystone et al. 2007). Iprodione inhibits several pathogens because it has a broad spectrum (Wei et al. 2020) and, among others, can hinder the growth of *Monilinia fructicola* (Thomidis et al. 2009).

Based on its constituent compounds, propiconazole belongs to the triazole fungicide, which inhibits fungal growth by inhibiting sterol biosynthesis, an essential

component in fungal cell defense (Rouabhi 2010). Propiconazole can inhibit the growth of *C. beticola* (Hudec et al. 2020) and *A. helianthi* (Usha et al. 2019). Evaluated for mycelial growth and conidial germination can determine the sensitivity of pathogenic fungi to fungicides. Sensitivity is a condition where the fungus is sensitive to a fungicide and reacts by inhibiting its growth. The growth of colony diameter indicates the sensitivity of the fungus to fungicides. The aim of this study was to conduct an in vitro test on the effect of fungicides, such as benomyl, carbendazim, mancozeb, iprodione, and propiconazole, on the mycelial growth and germination of conidia of *Alternaria* species causing leaf spot on sunflower.

MATERIALS AND METHODS

Procedures

Fungicides and fungal material

The fungicide bioassay experiment was conducted at the Laboratory of Mycology of the Faculty of Biology, Universitas Jenderal Sudirman, Indonesia. Five commercial fungicides, including: Masalgin 50WP (benomyl), Bendas 50WP (carbendazim), Dithane M-45 80WP (mancozeb), Rovral 50WP (iprodione), and Remazole 490EC (propiconazole) were obtained from pesticide distributor used in the experiment. Isolates of *Alternaria* sp. were isolated from sunflowers, and pure cultures were maintained on a PDA medium. As per the manufacturer's specifications, a stock solution of the fungicides was prepared by dissolving them in distilled water. The first factor was the type of fungicide, namely benomyl, mancozeb, iprodione, carbendazim, and propiconazole. The second factor was the concentration level of the (0%, 25%, 50%, 75%, 100%, and 125%) recommended dose. Furthermore, from the stock solution, different concentrations (0%, 25%, 50%, 75%, 100%, and 125%) of each fungicide were prepared, in which fungicide at 0% served as a negative control.

Mycelial growth sensitivity test

The prepared fungicide solution was mixed with 20 mL of PDA media and poured into a 9 cm diameter Petri dish (Ogolla et al. 2021). A fungal disc of 5 mm diameter was placed in the center of the petri dish and incubated at room temperature for 12 days. The control treatment was made without using fungicides. The entire procedure was done under aseptic conditions in Laminar Air Flow. Measurement of the diameter (in millimeters) of mycelial growth was taken 12 days after incubation. The Relative Inhibition Level (RIL) of the colony diameter was calculated using the following formula (Catao et al. 2013):

$$\%\mathbf{RIL} = \frac{\mathbf{d1}-\mathbf{d2}}{\mathbf{d1}} \times \mathbf{100\%})$$

Where:
d1: colony diametre of control
d2: colony diametre of treatment

Conidial germination sensitivity test

A drop of conidial suspension of each fungicide concentration was kept on a glass slide for conidial germination. Conidial germination was observed under a microscope after 24 hours of incubation at room temperature. One hundred conidia were observed per fungicide concentration. The treatment sensitivity level calculation was based on the relative resistance level of the fungicide to conidia germination in a medium mixed with fungicides. The percentage of Relative Inhibition Level (RIL) of conidial germination was calculated using the following formula (Catao et al. 2013):

$$\mathbf{RIL} = \frac{\sum \mathbf{control} - \sum \mathbf{treatment}}{\sum \mathbf{control}} \times \mathbf{100\%}$$

Where:
RIL: Relative Inhibition Level (%)
∑Control: number of germinated conidia in the control
∑Treatment: number of conidia grew in each treatment

Data analysis

The data obtained were analyzed by analysis of variance using the software SPSS at a 95% confidence level, followed by Duncan's test at a 95% confidence level (Steel and Torie 1995).

RESULTS AND DISCUSSION

The propiconazole and iprodione fungicides showed the highest (94.05% each) inhibition of mycelial growth, followed by mancozeb (82.14%), carbendazim (70.24%), and benomyl (59.76%). Meanwhile, the lowest mycelia growth inhibition was observed by the fungicide benomyl and carbendazim (29.28% each) (Table 1). This finding is aligned with Mahapatra and Das (2016), who reported iprodione and propiconazole could control *Alternaria* sp. in India. Furthermore, our findings differ from the findings of Desmukh et al. (2020) that the fungicide mancozeb is most effective in inhibiting *Alternaria* sp.. Prathuangwong et al. (1991) found that benomyl had the lowest ability to control *Alternaria* sp.. Vighe et al. (2018) reported that propiconazole is most effective in inhibiting the mycelium growth of *Alternaria* sp.. In comparison, mancozeb can inhibit the sporulation and germination of conidia *Alternaria* sp.. Ogolla et al. (2021) also reported that the fungicide carbendazim had the lowest inhibitory ability.

The highest (100%) inhibition of spore germination was recorded by fungicides propiconazole, iprodione, mancozeb, and carbendazim. Fungicide benomyl showed 97.0% inhibition of conidia germination (Table 2). Results showed that tested fungicides could inhibit the conidial germination of *Alternaria* sp. Our findings are in line with the findings of Koka et al. (2021) reported that the fungicide carbendazim was able to inhibit mycelium growth and conidia germination *Alternaria* sp..

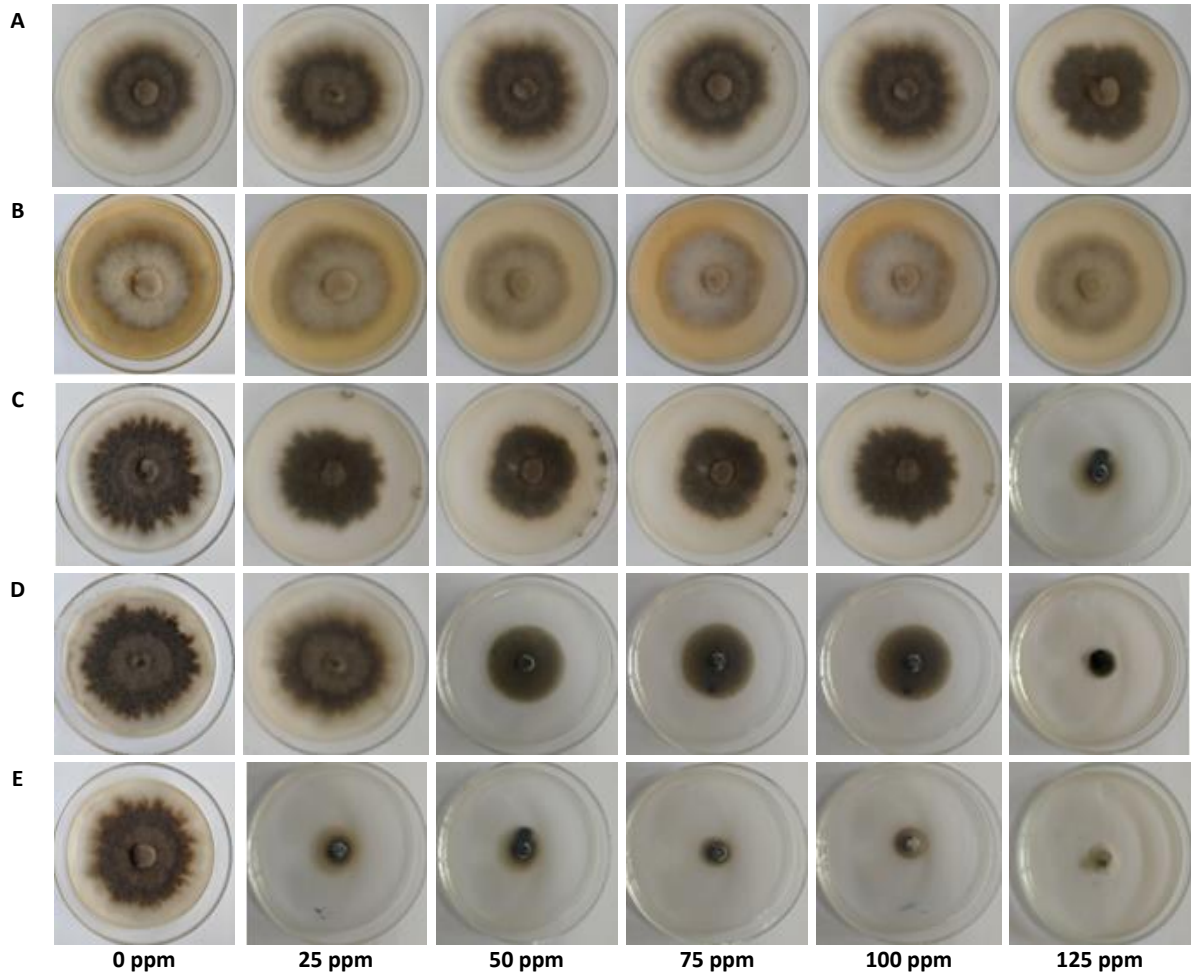


Figure 1. Mycelial growth of *Alternaria* sp. on several fungicides with various concentrations. A. Benomyl, B. Carbendazim, C. Mancozeb, D. Iprodione, and E. Propiconazole

Table 1. Percentage of inhibition of several synthetic fungicides on the mycelium growth of *Alternaria* sp.

Fungicides Dosage (%)	Benomyl	Carben-dazim	Mancozeb	Iprodi-one	Propicona-zole
0	0 VR	0 VR	0 VR	0 VR	0 VR
25	38.56 VR	29.28 VR	76.19 S	70 RS	94.05 VS
50	42.24 R	36.9VSR	76.92 S	71.43 MR	94.05 VS
75	52.38 R	44.05 R	77.62 S	73.81 MR	94.05 VS
100	54.28 R	61.9 MR	77.86 S	82.85 S	94.05 VS
125	59.76 R	70.24MR	82.14 S	94,05 VS	94,05 VS

Note: VR: Very Resistant, IR: Intermediate level of Resistance, R: Resistant, S: Sensitive, VS: Very Sensitive

Table 2. Percentage of inhibition of several fungicides on spore germination of *Alternaria* sp.

Fungicides Dosage (%)	Benomyl	Carben-dazim	Mancozeb	Iprodi-one	Propicona-zole
0	0 VR	0 VR	0 VR	0 VR	0 VR
25	89.60 VS	97.60 VS	98.20 VS	97.60 VS	95.40 VS
50	91.20 VS	100 VS	98.60 VS	98.40 VS	97.80 VS
75	92.40 VS	100 VS	100 VS	100 VS	100 VS
100	96.20 VS	100 VS	100 VS	100 VS	100 VS
125	97.00 VS	100 VS	100 VS	100 VS	100 VS

Note: VR: Very Resistant, IR: Intermediate level of Resistance, R: Resistant, S: Sensitive, VS: Very Sensitive

Table 3. Relative Inhibition Level (RIL) of fungicides on mycelium growth and spore germination of *Alternaria* sp.

Fungicides	Mycelial growth inhibition (%)	Spore germination inhibition (%)
Benomyl	40.32 b	77.73 a
Carbendazim	32,98 a	82.93 a
Mancozeb	62.59 c	82.80 a
Iprodione	65.38 c	82.67a
Propiconazole	78.38 d	82.20 a

Note: The number followed by the same letter in the same column is not significantly different at the 5% level (P>0.5)

The application of fungicides to the culture medium of *Alternaria* sp affected the growth of the mycelium of the fungus. The difference in the fungicide concentration also affects the mycelium's growth. The highest inhibition of mycelium growth *Alternaria* sp. was by propiconazole fungicide. While the lowest inhibition was by the fungicides benomyl and carbendazim (Figure 1). The relative-inhibition fungicides on mycelium growth was propiconazole (78.38%), followed by iprodione and mancozeb at 65.38% and 62.59%, respectively. The lowest (40.32%) mycelium growth inhibition was recorded by benomyl fungicide.

There is no difference in the inhibition ranges of fungicides against spore germination, indicating that they were all in the very sensitive range, which means that administration of all types of fungicides had a very high ability to inhibit spore germination of *Alternaria* sp. (Table 3). This finding is in line with Vighe et al. (2018), which stated that propiconazole was most effective in controlling *Alternaria cassia*. In addition, van den Berg et al. (2002) stated that carbendazim and propiconazole had the same ability to inhibit the mycelium growth of *Alternaria* sp.. This finding is also in line with Yang et al. (2011), who stated that fungicides could inhibit sporulation, spore germination, and germination of germ tubes. Those effects are due to interference in the osmoregulatory signal transmission pathway, thereby inhibiting hyphae formation.

Differences in the ability of fungicides to inhibit mycelium growth and germination of *Alternaria* sp. spores were due to the different activities of each fungicide. Benomyl and carbendazim affect mitosis and cell division, namely tubulin polymerization into microtubules. Rouabhi (2010) stated microtubules are cytoskeletal polymers in eukaryotic cells and play important roles in many cellular functions. Mancozeb has multi-site activities. Multisite-activity fungicides are widely used in agronomic activities because of their broad spectrum of disease control activity. Still, they may have side effects on other microorganisms due to the impact of their various biochemical sites. Mancozeb affects the metabolism of target microorganisms. Iprodione has the activity of inhibiting glycerol synthesis and hyphae development by cutting signal transduction. In addition, propiconazole inhibits Demethylation (DMI) and sterol biosynthesis in fungal cells. Sterols are another important component of cell membranes in fungi.

In addition to differences in activity, differences in the ability of fungicides to inhibit mycelium growth and spore germination may be influenced by the genetic conditions of pathogenic fungi. Genetic differences possessed by pathogenic fungi can cause differences in sensitivity because genetic diversity affects the ability of fungi to survive and adapt to active fungicide applications. The emergence and development of resistant pathogens can be caused by repeated use of fungicides over a long period in a season, systemic fungicides, and continuous use at doses above lethal concentrations (van Tuyl 1977). Each fungicide has a different way of working in inhibiting pathogenic fungi. Systemic fungicides work simultaneously with plant metabolic processes and only act on one site of fungal cells, so they are said to have a single site or specific mechanism of action. Fungicides with a single-site mechanism of action generally have a high risk of developing fungal resistance to this active ingredient more rapidly than fungicides with a multi-site mechanism of action (Secor and Rivera 2012).

The application of fungicides to control hinders the growth of pathogenic fungi, and in some instances, the pathogen tries to adapt continuously, causing resistance to the given fungicide (Yang et al. 2019). The mechanism of the strains resistant to several fungicides occurs due to a decrease in the permeability of pathogenic cells to absorb chemical compounds. The mechanism for the emergence of strains resistant to several fungicides is a decrease in the permeability of pathogenic cells to absorb chemical compounds, detoxification of chemical compounds by pathogenic cells, decreased conversion to more toxic metabolites, decreased affinity for pathogenic cells, and by-passing. In addition, the sequence of reactions in metabolic processes and the production of replacement enzymes is inhibited by treatment with chemical compounds (Agrios 2005). The differences in the sensitivity level of *Alternaria* sp. to all fungicides were caused by the mode of action of each active ingredient of the fungicide, the level of fungicide toxicity to pathogens, the method of use carried out by farmers, and the genetic diversity of the two species of pathogenic fungi.

Application of fungicides following the recommended dose on the fungal growth medium affected the sensitivity level of tested fungi. The level of sensitivity of fungi to fungicides can be known from differences in the growth of colony diameters. Based on test results of inhibition of mycelium growth in each treatment, there were differences in the size of the colony diameter. Therefore, a more effective fungicide inhibits the growth of fungal mycelium. Vighe et al. (2018) stated that propiconazole effectively inhibits mycelium growth, sporulation, and conidial germination of *A. cassiae*. This study revealed that *Alternaria* species were sensitive to mancozeb and propiconazole but resistant to benomyl, carbendazim, and iprodione. In addition, it has also been observed that fungicides mancozeb and propiconazole effectively controlled the pathogens.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the BLU Universitas Jenderal Soedirman (Unsoed), Purwokerto Utara, Indonesia, for allowing them to conduct this research under *Riset Dasar Unsoed* (RDU) Scheme Number 1068/UN23/HK 02/2021. The authors would also like to thank the Head of the Mycology Laboratory of the Faculty of Biology, Universitas Jenderal Soedirman for their technical support in this research.

REFERENCES

Aalum K, Ahmad I, Kumar B, Singh SD, Ganguly S. 2016. Appliance of fungicide on the *Alternaria* leaf spot disease of red delicious apple (*Malus domestica* Borkh) caused by the pathogen *Alternaria mali*. Intl J Innov Res Sci 5 (7): 12747-12752. DOI: 10.15680/IJIRSET.2016.0507124.

Adeleke BS, Babalola OO. 2020. Oilseed crop sunflower (*Helianthus annuus*) as a source of food: Nutritional and food benefits. Food Sci Nut 8: 4666-4684. DOI: 10.1002/fsn3.1783.

Agrios GN. 2005. Plant Pathology, 4th ed. Elsevier, London.

Allen PM, Gottlieb D. 1970. Mechanism of action of the fungicide thiabendazole 2-(4'-thiazolyl) benzimidazole. Appl Microbiol 6: 919-926. DOI: 10.1128/am.20.6.919-926.1970.

Bianchini A, Bullerman LB. 2014. Mycotoxins Classification. In: Batt CA, Tortorello ML (eds.). Encyclopedia of Food Microbiology, 2nd ed. Academic Press, New York. DOI: 10.1016/B978-0-12-384730-0.00230-5.

Bitar MC, Lafarge N, Sok P, Cayot, Bou-Maroun E. 2019. Molecularly imprinted sol-gel polymers for the analysis of iprodione fungicide in wine: synthesis in green solvent. Food Chem 293 (1): 226-232. DOI: 10.1016/j.foodchem.2019.04.108.

Blystone CR, Lambright CS, Furr J, Wilson VS, EarlGray L. 2007. Iprodione delays male rat pubertal development, reduces serum testosterone levels, and decreases ex vivo testicular testosterone production. Toxicol Lett 174 (1-3): 74-81. DOI: 10.1016/j.toxlet.2007.08.010.

Catao HCRM, Sales NDLP, Azevedo DMQ, Flavio NSDD, Menezes JBDS, Barbosa LV, Martinez RAS. 2013. Fungicides and alternative products in the mycelial growth and germination control of *Alternaria tomatophila*. Idesia (Chile) 31 (3): 21-28. DOI: 10.4067/S0718-34292013000300004.

Chandrakala J, Vidyasagar B Rajanikanth P. 2018. In-vitro evaluation of different fungicides against chilli twig blight caused by *Choanephora cucurbitarum*. Intl J Pure Appl Biosci 6 (3): 715-718. DOI: 10.18782/2320-7051.6916.

Cho HS, Yu SH. 2000. Three *Alternaria* species pathogenic to sunflower. Plant Pathol J 16 (6): 331-334.

Desmukh HV, Deokar CD, Raghuwanshi KS, Khaire PB, Brahmane PR. 2020. Efficacy of different fungicides against the *Alternaria solani* under in vitro conditions. J Pharmacogn Phytochem 9 (6): 1057-1060.

Devi PA, Paramasivam M, Prakasam V. 2015. Degradation pattern and risk assessment of carbendazim and mancozeb in mango fruits. Environ Monit Assess 187 (1): 4142. DOI: 10.1007/s10661-014-4142-6.

Hudec K, Mihók M, Roháčik T, Mišľan L. 2020. Sensitivity of *Cercospora beticola* to fungicides in Slovakia. Acta Fytotechn Zootechn 23 (3): 147-154. DOI: 10.15414/afz.2020.23.03.147-154.

Jena RK, Swain NC, Behera SK, Jena NK, Rout M, Pratap DB, Mohapatra A, Nayak BK. 2020. Environmental challenges on leaf spot and blight of sunflower (*Helianthus annuus*, L). Intl J Curr Microbiol App Sci 9 (7): 2756-2765. DOI: 10.20546/ijcmas.2020.907.325.

Koka JA, Bhat MY, Wani AH. 2021. In vitro efficacy of fungicides on mycelial growth and spore germination of *Alternaria alternata* and *Mucor plumbeus*. J Drug Deliv Therapeut 11 (3): 17-22. DOI: 10.22270/jddt.v11i3.4692.

Ogolla FO, Nyakiniwa R, Chabari SK, Onyango BO. 2021. In-vitro evaluation of fungicide sensitivity of tomato, leaf blight pathogens. J Pertanian Trop 8 (1): 11-25. [Indonesian]

Ons L, Bylemans D, Thevissen K, Cammue BPA. 2020. Combining biocontrol agents with chemical fungicides for integrated plant fungal disease control. Microorganisms 8 (12): 1930. DOI: 10.3390/microorganisms8121930

Prathuangwong. S, Kao SW, Sommartya T, Sinchaisri P. 1991. Role of four *Alternaria* spp causing leaf and stem blight of sunflower in Thailand and their chemical control. Kasetsart J (Nat Sci) 25: 112-124.

Purwati RD, Herwati A. 2016. Evaluation of quantitative and qualitative morphological characters of sunflower (*Helianthus annuus*) germplasm. Biodiversitas 17: 461-465. DOI: 10.13057/biodiv/d170209.

Rouabhi R. 2010. Introduction and Toxicology of Fungicides. In: Carisse O (eds). Fungicides Ed. Intech Open LTD., London. DOI: 10.5772/12967.

Secor GA, Rivera VV. 2012. Fungicide resistance assays for fungal plant pathogens. In: Bolton M, Thomma B (eds). Plant Fungal Pathogens. Methods in Molecular Biology (Methods and Protocols) 835. Humana Press, New Jersey. DOI: 10.1007/978-1-61779-501-5_23.

Steel RGD, Torrie JH. 1995. Prinsip dan Prosedur Statistik. Penerjemah: Sumantri B. PT. Gramedia Pustaka Utama, Jakarta. [Indonesian]

Thomidis T, Michailides T, Exadaktylou E. 2009. Contribution of pathogens to peach fruit rot in northern Greece and their sensitivity to iprodione, Carbendazim, thiophanate-methyl, and Tebuconazole Fungicides. J Phytopathol 157 (1): 194-200. DOI: 10.1111/j.1439-0434.2008.01469.x.

Usha D, Prasad SML, Sarada C. 2019. Sensitivity of different fungicides against isolates of *Alternariaster helianthi* (Hansf) Tubaki and Nishihara, leaf blight in sunflower. Indian Phytopathol 72 (2): 351-360. DOI: 10.1007/s42360-018-00109-2.

Utlwang R, Khare R, Khonga KB, Segwagwe EB, Thethe A. 2017. Assessing the effectiveness of locally available fungicides in managing Cercospora leaf spot Swiss chard under field conditions in Botswana. Intl J Food Agric Vet Sci 7 (1): 1-8.

Van den Berg. N, Aveling TAS, Venter SL. 2002. The evaluation of six fungicides for reducing *Alternaria cassia* on cowpea seed. Crop Protect 21: 501-505. DOI: 10.1016/S0261-2194(01)00140-5.

Van Tuyl JM. 1977. Genetics of Fungal Resistance to Systemic Fungicides. Van de Landbouwhogeschool, Wageningen.

Vighe CS, Nema S, Chobe DR, Thakare GA. 2018. Effect of fungicides against the mycelial growth, sporulation, and spore germination, of *Alternaria cassia* causing *Alternaria* leaf blight of cowpea. Intl J Chem Stud 6 (1): 77-79.

Viriyasuthee W, Saksirirat W, Saepaisan S, Gleasson M, Jogloy S. 2019. Variability of *Alternaria* leaf spot resistance in Jerusalem Artichoke (*Helianthus tuberosus* L.) accessions grown in a humid tropical region. Agronomy 9 (268): 1-8. DOI: 10.3390/agronomy9060268.

Wei Y, Meng Y, Huang T, Liu Z, Zhong K, Ma J, Zhang W, Li Y, Lu H. 2020. Development toxicity and cardiotoxicity in zebrafish from exposure to iprodione. Chemosphere 263 (1): 127860. DOI: 10.1016/j.chemosphere.2020.127860.

Yang C, Hamel C, Vujanovic V, Gan Y. 2011. Review article fungicide: Modes of action and possible impact on nontarget microorganisms. Intl Schol Res Net Ecol 2011: 1-8. DOI: 10.5402/2011/130289.

Yang LN, He MH, Ouyang HB, Zhu W, Pan ZC, Sui QJ, Zhan J. 2019. Cross-resistance of the pathogenic fungus *Alternaria alternata* to fungicides with different modes of action. BMC Microbiol 19 (1): 1-10. DOI: 10.1186/s12866-019-1574-8.

Zhang Y, Yu Y, Jia R, Liu L, Zhao J. 2021. Occurrence of *Alternaria* leaf blight of sunflower caused by two closely related species *Alternaria solani* and *A. tomatophila* in Inner Mongolia. Oil Crop Sci 6: 74-80. DOI: 10.1016/j.ocsci.2021.04.006.

ASIAN JOURNAL OF AGRICULTURE
Volume 7, Number 1, June 2023
Pages: 52-56

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g070107

Molecular characterization of filamentous fungi associated with spoilage of sweet oranges sold in Choba Market, Port Harcourt, Nigeria

CHINYERUM GLORIA IKECHI-NWOGU♥, BLESSING ADANTA ODOGWU, MARIA ADANIMA EDUMASAM
Department of Plant Science & Biotechnology, Faculty of Science, University of Port Harcourt. Port Harcourt 500001, Rivers State, Nigeria.
Tel.: +234-8035426615, ♥email: chinyerum.nwogu@yahoo.com

Manuscript received: 11 October 2022. Revision accepted: 22 February 2023.

Abstract. *Ikechi-Nwogu CG, Odogwu BA, Edumasam MA. 2023. Molecular characterization of filamentous fungi associated with spoilage of sweet oranges sold in Choba Market, Port Harcourt, Nigeria. Asian J Agric 7: 52-56.* Post-harvest spoilage of sweet oranges is one of the major causes of post-harvest losses. This study was conducted to identify the fungi associated with post-harvest spoilage of sweet oranges using molecular techniques in Choba Markets, Rivers State, Nigeria. The DNA of the fungal isolate SO-1 and SO-2 were characterized using Internal Transcribed Spacer 4 and 5 molecular markers and aligned by the Basic Local Alignment Search Tool for Nucleotide (BLASTN) 2.8.0 of National Centre for Biotechnology Information (NCBI) database. Based on the sequence similarities, it was observed that isolate SO-1 was 98.96% identical to *Neurospora crassa* Shear & B.O.Dodge while SO-2 was 99.48% identical to *Aspergillus flavus* Link. These findings showed that *N. crassa* and *A. flavus* are some of the causal fungal pathogens of spoilt sweet oranges. It is anticipated that this result will provide information that will be helpful in the deployment of the appropriate post-harvest management of these fungi during post-harvest handling to minimize the spoilage of sweet oranges and thus reduce post-harvest losses.

Keywords: *Aspergillus flavus,* Choba Market, *Neurospora crassa,* RBCL marker, spoilage, sweet orange

INTRODUCTION

Fruits are the edible part of flowering plants' developed ovaries, which are generally eaten raw (Uji 2007; Ikhiwili 2012). Fruits are an important class of food needed for normal growth and development; humans and animals depend on them. Aside from humans, organisms such as bacteria and fungi also rely on fruits as a food source (Haase 2008). High sugars and low pH values make fruits desirable for fungal spoilage (Kuyu and Tola 2018).

In botany, an orange is referred to as any small, evergreen tree or shrub bearing round fruits (Daubenton 2013). However, it is called sweet orange to differentiate it from the *Citrus aurantium* Lour., referred to as bitter orange. Sweet orange is botanically known as *Citrus sinensis* (L.) Osbeck is one of Nigeria's main money making fruits (Gorinstein et al. 2001).

Sweet orange belongs to the family Rutaceae because they are often characterized as trees and shrubs and have strong scents. They appear to be one of the major commercial fruit crops that are widely eaten fresh, processed into juices or peels used as a fragrance. It is a principal source of vitamin C; nevertheless, once cut or squeezed, it speedily begins to be dispensed. After only eight hours at room temperature or 24 hours in a refrigerator, there is about a 20 percent loss of vitamin C (Pathy 2018). Moreover, oranges are a good source of folate, a source of vitamins A and B1, and dietary fiber (Kubala and Arnarson 2021). It comprises calcium, potassium, carotenoids, magnesium, flavonoids, antioxidants, acids, essential oils, folate, iron, manganese, zinc, sodium, and iodine (Dhalaria et al. 2020). Some other products made from oranges include: orange oil, orange tea, orange blossom honey, marmalade, and slug repellent. According to Barros et al. (2012), Taylor et al. (2020), and Marcene (2021), sweet oranges have been reported to have anti-inflammatory, and anti-tumor properties, prevent cancer, asthma, obesity, arthritis, kidney stones, coronary heart diseases, high blood pressure, and stroke, and help maintain healthy mucus membranes, skin, and vision. It also boosts our immune system and helps our body to fight diseases. In addition, various fruits have naturally occurring polyphenolic compounds (Nile and Park 2014) that function as free radical inhibitors and play vital roles in preventing aging (Arif et al. 2022) and their associated diseases.

Orange has a thick, bitter rind that can be processed into animal feed. It is also used as a flavoring or for garnishing. The white portion of the rind (pericarp or albedo) is a source of pectin and has almost the same amount of vitamin C as the flesh. The crop is mainly cultivated in subtropical and tropical regions (Encyclopedia of Food and Culture 2022), globally in over 137 countries on six continents. In Nigeria, its production spreads throughout the country.

Even though sweet orange encompasses numerous components which are beneficial to man, fungi affect their economic, medicinal, traditional, and nutritional value, thereby shortening their shelf life. In developing countries, where protection and handling of fresh fruit are inadequate, losses during storage and transit can represent the harvested crop by over 50% (Eckert and Ogawa 1985).

When fruits become less palatable, and there is a change in the usual taste, it indicates that pathogens have

invaded the fruits and changed their natural conditions. Aside from the alterations of taste, there are texture, smell, and physical appearance changes owing to the actions of pathogen invasion. These fungi are known tof destroy fruits, reducing consumption and income. Therefore, microorganisms need to be identified to reduce the risk of contamination and infection from handling and consuming these fruits. The objective of this research was to isolate and classify the filamentous fungi associated with the spoilage of sweet orange fruits sold in Choba Market, Port Harcourt, to reduce losses.

Orthodox approaches have been used to identify pathogens; however, according to Lutzoni et al. (2004), these methods are bulky and inefficient. Furthermore, morphological characters can be contentious or problematic even for trained mycologists, as they may not always provide accurate groupings within an evolutionary framework, mainly at the species level. Effective identification of disease-causing fungal pathogens of sweet orange is proper because, according to St-Germain and Summerbell (2003), diseases caused by fungi have become a noteworthy medical problem and are increasing at a disturbing rate. In addition, the increase in the number of patients that are not immuno-competent has emphasized the significance of the precise identification of fungi. Therefore, to accurately identify these pathogens, a molecular characterization technique has been employed to verify the identification of the fungi pathogens (Gontia-Mishra et al. 2013; Bechem and Afanga, 2017).

MATERIALS AND METHODS

Study area and sample collection

The study was conducted at the Regional Centre for Biotechnology and Bioresources Research, University of Port Harcourt, Choba, Rivers State, Nigeria. Polymerase Chain Reaction (PCR) product sequencing was done at the International Institute for Tropical Agriculture (IITA) in Ibadan, Nigeria. Twenty (20) fresh samples with infected parts were collected randomly from the Choba Market in Port Harcourt, Rivers State, Nigeria, in February 2020. The samples were collected into sample bags, labeled, and immediately taken to the laboratory.

Isolation of fungi from sweet orange using the blotter method

The modified standard blotter method of Ikechi-Nwogu et al. (2019) was used to isolate fungi associated with sweet oranges. The filter paper, distilled water (placed in a conical flask), and petri dishes (wrapped in foil paper) used for the work was first autoclaved at 121°C for 15 minutes. Next, the petri dishes were lined with three layers of sterilized 9 cm Whatman's filter paper, the filter paper was soaked with little water, and then the petri dishes were covered immediately. Next, four (4) small bud clusters were surface sterilized in a beaker (Plate 2.1) using 70% ethanol for 2-3 minutes. Next, the ethanol was discarded, rinsed with sterile distilled water twice, and plated per petri dish. Then the plates were wrapped with masking tape and then labeled. After this, they were incubated at 25 + 2°C in the laboratory for seven days. Finally, all identified fungi were sub-cultured on Potato Dextrose Agar (PDA) medium under darkness at room temperature (25+ 2°C).

Morphological and microscopic identification and characterization

The morphological identification of isolates KN-01 was conducted by visually observing the mycelium and comparing their colonies for their diameters, colors of conidia, reverse colors, and texture. The isolates were later subjected to microscopic analysis for identification by an electron binocular microscope at X40.

Molecular characterization using the Internal Transcribed Spacer (ITS) marker and identification

The Genomic DNA of the two isolates SO-1 and SO-2 found on the oranges were extracted following the protocol of Quick-DNATM Fungal/Bacterial MiniPrepKit (Zymo Research Group, California, USA). The manufacturer described the protocol by modifications at the Regional Center for Biotechnology and Bioresources (RCBB), the University of Port Harcourt, Choba, Rivers State, Nigeria. The isolate DNA quantity and concentration were measured using Nano-Drop 2000c spectrophotometer (Thermo Fisher Scientific Inc. Wilmington, Delaware, USA). The DNA purity was measured as a ratio of absorbance at 280 nm to that of 260 nm. The DNA samples were sent to the International Institute of Tropical Agriculture (IITA) Bioscience Center, Ibadan, Nigeria, for sequencing and amplification. Furthermore, the primers used to amplify the nuclear ribosomal DNA (rDNA) fragments of the SO-1 and SO-2 isolates were the Internal Transcribed Spacer 4 (ITS4) with the sequence TCCTCCGCTTATTGATATGS and ITS5 with the sequence GGAAGTAAAAGTCGTAACAAGG. The ABI 3500 capillary electrophoresis sequencer sequenced the amplicons. Then, the DNA sequence file was saved in the Bioedit file with an extension. ab1. The Molecular Evolutionary Genetics Analysis (MEGA) version 7.0.26 software analyzed the sequence. Next, the sequence was aligned by the Basic Local Alignment Search Tool for nucleotide (BLASTN) 2.8.0 version to the database of the National Center for Biotechnology Information (NCBI).

Pathogenicity tests of the fungi

Healthy orange fruits were inoculated with each fungus to assess their ability to induce spoilage on the healthy oranges. That was achieved using the methods described by Chukwuka et al. (2010). Twenty healthy oranges were washed and rinsed with distilled water and surface-sterilized using 70% ethanol. A sterilized cork borer was used to bore holes in each of the fruits to enable inoculation. The holes were sealed with petroleum jelly to avoid infection. Afterward, the fruits were each placed in sterilized polythene bags, incubated at 25 + 2°C in the laboratory, and observed for five days. When symptoms developed and were followed by the appearance of visible fungi, the fungi were sub-cultured from the inoculated fruits and compared with the original isolates.

Morphological and microscopic characterization and identification

The morphological identification of the isolates was conducted by visually observing the mycelium and comparing their colonies for their diameters, colors of conidia, reverse colors, and texture. The isolates were later subjected to microscopic analysis for identification using an electron binocular microscope at X40.

RESULTS AND DISCUSSION

Morphological identification

The post-harvest shelf-life of fresh fruits is one limiting factor affecting their economic value. However, during post-harvest handling in developed and developing countries, about 20-25% of harvested fruits are decomposed by pathogens (Zhu 2006; Strano et al. 2017). Fungal infection of fruits, such as sweet oranges (*C. sinensis*), generally occurs during harvest and growing seasons, handling, transportation, storage, marketing, or after purchase by the consumer (Yusuf et al. 2022). Several organisms have been associated with the post-harvest decay of sweet oranges. Among the organisms are *Aspergillus fumigatus*, *Aspergillus niger*, *Rhizopus stolonifer*, *Neurospora crassa*, and *Aspergillus flavus* (Tafinta et al. 2013).

In this study, two fungal organisms coded SO-1 and SO-2 were isolated and found to be associated with sweet oranges sold in the Choba Market. From the morphological visual observation, the isolates SO-2 (Figure 1 *left*) developed powdery yellowish-green spores on the surface and a reddish-gold colored mycelium at the reverse side of the Petri dish, which indicated that it was similar to *Aspergillus* spp. At the same time, SO-1 (Figure 1 *right*) produced orange-colored spores that are both visible to the naked eye, similar to *Neurospora* spp. (Figure 1).

Molecular characterization using the Internal Transcribed Spacer (ITS) marker and identification

The molecular study was conducted to determine the specific species of the fungal genera *Neurospora* spp. and *Aspergillus* spp., and the genomic DNA of the isolates SO-1 and SO-2 were successfully extracted. The Nano Drop result showed that the isolates' DNA concentrations were 130.3ng/µL and 128.7ng/µL. While the absorption peak of the 260nm/280nm readings was 1.387 and the 260nm/230nm readings 1.357, respectively (Table 1).

Polymerase chain reaction (PCR)

The result of the amplified DNA band of the isolates SO-1 and SO-2 are presented in Figure 2. From the result, the SO-1 and SO-2 isolates sequence had over 587 and 600 base pairs, respectively.

The SO-1 and SO-2 isolate sequences aligned with 100 sequences deposited in the National Center Biotechnology Information (NCBI) composite biological database. The SO-1 isolate sequence was 98.96% identical to *N. crassa* (MH790549.1), while SO-2 was 99.48% identical to *A. flavus* (MH591447.1). The phylogenetic tree result showed the relationship between the isolates SO-1 from this study and other fungal isolates on the NCBI database is shown in Figures 3A and 3B. In addition, the phylogenetic analysis showed that SO-1 was related to other *Neurospora* species, such as *N. tetrasperma, N. intermeda,* and *N. dictyophora.*

Table 1. The concentration of DNA extracted from fungal isolates of sweet oranges using a Nano-drop (2000c) spectrophotometer

Fungi sample ID	DNA concentration (ng/µL)	Absorbance at 260nm/280nm (purity)
SO-1	130.3	1.387
SO-2	128.7	1.357

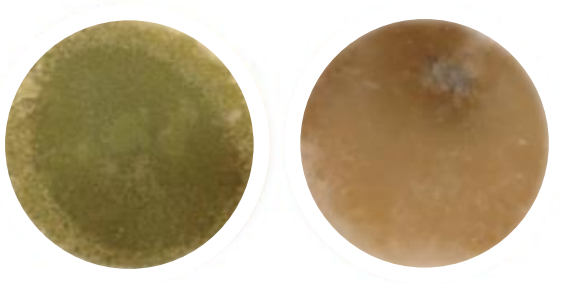


Figure 1. Pure culture of fungus isolated from sweet orange, S0-2 (*left*) and SO-1 (*right*)

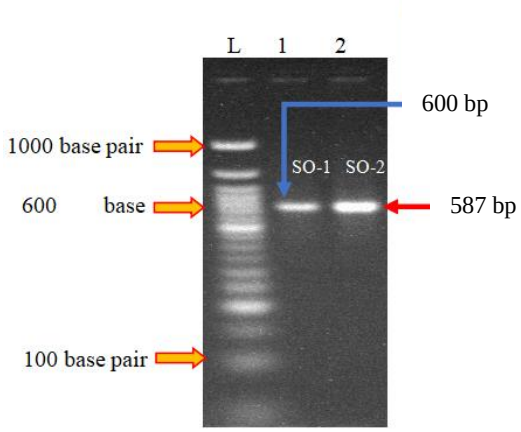


Figure 2. Amplified PCR product generated from SO-1 and SO-2 isolates using ITS marker. L: DNA Ladder, L=1 KB Ladder Sequence Alignment

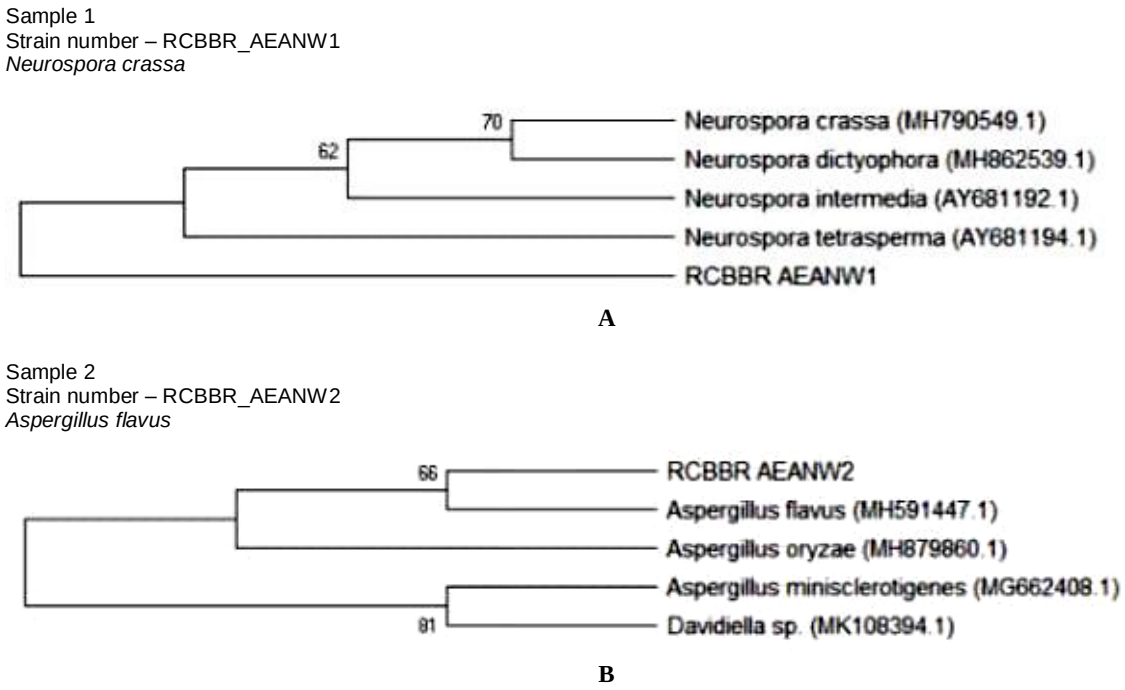


Figure 3. Phylogenetic tree generated by maximum composite likelihood analysis based on the ITS 4-5 gene sequences. A. *Neurospora crassa*, B. *Aspergillus flavus*

The identification of *Aspergillus* spp. as a causal pathogen for post-harvest rot in sweet oranges is similar to the work by Tafinta et al. (2013) and Oviasogie et al. (2015). They reported that *Aspergillus* spp. is the principal organism associated with the post-harvest rot of sweet oranges and that they produce several toxic metabolites like aflatoxins that are hazardous to human health. The use of molecular techniques was pertinent in elucidating the specific fungal species causing the rot of sweet oranges. Molecular techniques have proven more dependable than traditional methods as they allow comparing DNA sequence information between known and unknown fungal species DNA sequences from public repositories. The morphological (traditional) description using visual observation of the spores and mycelium commonly used for identifying fungi has led to the wrong identification of fungal isolates (Ikechi-Nwogu et al., 2019). The molecular techniques employed in this study led to the successful characterization of two (2) fungi isolated causing rot of sweet oranges, namely *A. flavus* and *N. crassa*, which belong to the division Magnoliophyta, class Magnoliopsida, order Sapindales, and family Rutaceae. *Aspergillus* species are producers of mycotoxins. When consumed, these mycotoxins are secondary metabolites harmful to animals and humans.

In conclusion, findings from the study identified *A. flavus* and *N. crassa* as the causal pathogens associated with post-harvest rot of sweet oranges using molecular techniques. Therefore, this technique's use in identifying the fungal pathogen is pertinent. Also, the identification of these pathogens will be helpful in the deployment of the appropriate post-harvest management and control of these fungi during post-harvest handling to minimize the spoilage of sweet oranges and thus reduce post-harvest losses.

ACKNOWLEDGEMENTS

We express our profound gratitude to the Department of Plant Science and Biotechnology, the staff at the Regional Centre for Biotechnology and Bioresources Research Laboratory, University of Port Harcourt, Choba, Rivers State, Nigeria, and the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria, for helping out with the DNA extraction and quality check.

REFERENCES

Arif MU, Khan MKI, Riaz S, Nazir A, Maan AA, Amin U, Saeed F, Afzaal M. 2022. Role of fruits in aging and age-related disorders. Exp Gerontol 162: 111763. DOI: 10.1016/j.exger.2022.111763.

Barros HR, Ferreira TA, Genovese MI. 2012. Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil. Food Chem 134 (4): 1892-1898. DOI: 10.1016/j.foodchem.2012.03.090.

Bechem ET, Afanga YA. 2017. Morphological and molecular identification of fungi associated with corm rot and blight symptoms on plantain (*Musa paradisiaca*) in macro-propagators. Intl J Biol Chem Sci 11 (6): 2793-2808. DOI: 10.4314/ijbcs.v11i6.19.

Chukwuka KS, Okonkon IO, Adekunle AA. 2010. Microbial ecology of organisms causing pawpaw (*Carica papaya* L.) fruit decay in Oyo State, Nigeria. Ame-Eur J Toxicol Sci 2 (1):43-50.

Daubenton P. (le Subdélégué). 2013. Orange Tree: The Encyclopedia of Diderot & d'Alembert Collaborative Translation Project. Michigan Publishing, United State America.

Dhalaria R, Verma R, Kumar D, Puri S, Tapwal A, Kumar V, Nepovimova E, Kuca K. 2020. Bioactive compounds of edible fruits

with their anti-aging properties: A comprehensive review to prolong human life. Antioxidants 9 (11): 1123. DOI:10.3390/antiox9111123.

Eckert JW, Ogawa JM. 1985. The chemical control of post harvest diseases: Subtropical and tropical fruits. Ann Rev Phytopath 23: 421-454. DOI: 10.1146/ANNUREV.PY.23.090185.002225.

Encyclopedia of Food and Culture 2022. Tropical and Subtropical Fruit. Encyclopedia.com: https://www.encyclopedia.com/food/encyclopedias-almanacs-transcripts-and-maps/tropical-and-subtropical-fruit

Gontia-Mishra I, Deshmukh D, Tripathi N, Bardiya-Bhurat K, Tantwai K, Tiwari S. 2013. Isolation, morphological and molecular characterization of phytate-hydrolysing Fungi by 18S rDNA sequence analysis. Braz J Microbiol 44 (1): 317-323. DOI: 10.1590/S1517-83822013005000021.

Gorinstein S, Martin-Belloso O, Park Y, Haruenkit, R, Lojek A, Ciz M, Capsi A, Libma I, Traktenberg S. 2001. Comparison of some biochemical characteristics of different *Citrus* fruits. Food Chem 74 (3): 309-315. DOI: 10.1016/S0308-8146(01)00157-1.

Haase J. 2008 What Do Fungi Eat? The 3 Main Diets of Fungi. https://a-z-animals.com/blog/what-do-fungi-eat-the-3-main-diets-of-fungi/ [2023 January 16].

Ikechi-Nwogu CG, Odogwu BA, Pikibo A. 2019. Morphological and molecular characterization of post-harvest fungal pathogen of ginger rhizomes. Niger J Mycol 11: 196-203.

Ikhiwili OM. 2012. Isolation and Characterisation of Microorganisms Associated with Rot Diseases of Fruit, Stem and Leaf of *Carica papaya* L. [Dissertation]. Covenant University, Canaanland, Ota, Ogun State. [Nigeria]

Kubala J, Arnarson A. 2021. Oranges: Nutrients, Benefits, Juice, and More. http://www.healthline.com/nutrition/oranges.

Kuyu CG, Tola Y. B. 2018. Assessment of banana fruit handling practices and associated fungal pathogens in Jimma Town Market, Southwest Ethiopia. Food Sci Nutrit 6 (3): 609-616. DOI: 10.1002/fsn3.591.

Lutzoni F, Kauff F, Cox CJ, McLaughlin D, Celio G, Dentinger B, Padamsee M, Hibbett D, James TY, Baloch E, Grube M, Reeb V, Hofstetter V, Schoch C, Arnold AE, Miadlikowska J, Spatafora J, Johnson D, Hambleton S, Crockett M, Vilgalys R. 2004. Assembling the fungal tree of life: Progress, classification, and evolution of subcellular traits. Am J Bot 91 (10): 1446-1480. DOI: 10.3732/ajb.91.10.1446.

Marcene B. 2021. 13 Amazing Health Benefits of Oranges. http://www.Naturalfoodseries.com/13 Benefits of Oranges. DOI: 10.31838/ijpr/2021.13.02.069.

Nile SH, Park SW. 2014. Edible berries: Bioactive components and their effect on human health. Nutrition 30: 134-144. DOI: 10.1016/j.nut.2013.04.007.

Oviasogie F, Ogofure AG, Beshiru A, Ode N. 2015. Assessment of fungal pathogens associated with orange spoilage. Afr J Microbiol Res 9 (29): 1758-1763. DOI: 10.5897/AJMR2014.7246.

Pathy K. 2018. Process for preparation of vitamin C and method for determination of vitamin C in tablets. SF J Chem Res 2 (1): 1-7. DOI: 10.32474/SCSOAJ.2018.01.000114.

St-Germain G, Summerbell R. 2003. Identifying Fungi: A Clinical Laboratory Handbook. Star Publishing Company, United State America. DOI: 10.1590/S0036-46652003000300017.

Strano MC, Altieri G, Admane N, FrancescoGenovese F, Renzo GCD. 2017. Advance in *Citrus* post-harvest management: Diseases, cold storage and quality evaluation. In: Gill H, Garg H (Eds.). *Citrus* Pathology. IntechOpen. DOI: 10: 66518. DOI: 10.5772/66518.

Tafinta IY, Shehu K, Abdulganiyyu H, Rabe A M, Usman A. 2013. Isolation and identification of fungi associated with the spoilage of sweet orange (*Citrus sinesis*) fruits in Sokoto State. Niger J Basic Appl Sci 2 (1): 15.

Tahan Uji. 2007. Species diversity of indigenous fruits in Indonesia and its potential. Biodiversitas 8 (2): 157-167. DOI: 10.13057/biodiv/d080217.

Taylor CW, Regan LB, Jeffrey BB, Britt B-F, Oliver C, Kristi MC-W, Adam D, Shirin H, Elizabeth J, Richard L, Robert M, Sue AS, Ding DW. 2020. Fruits, vegetables, and health: A comprehensive narrative, umbrella review of the science and recommendations for enhanced public policy to improve intake. Crit Rev Food Sci Nutr 60 (13): 2174-2211. DOI: 10.1080/10408398.2019.1632258.

Yusuf HZ, Muhammad A, Mukhtar MD. 2022. Point of marketing survey for fungi associated with spoilage of oranges distributed at some depots in Kano Nigeria. Bayero J Appl Sci 13 (1): 192-197. DOI: 10.4314/bajopas.v13i1.31S.

Zhu SJ. 2006. Non-chemical approaches to decay control in post-harvest fruit. In: Noureddine B, Norio S (Eds.). Advances in Post-harvest Technologies for Horticultural Crops. Research Signpost, India.

ASIAN JOURNAL OF AGRICULTURE
Volume 7, Number 1, June 2023
Pages: 57-68

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g070108

Biodiversity of edible fruit sold at Pasar Gede, Surakarta City, Central Java, Indonesia

DHEA GITA FITRI SAGITARIAN[1], LUNA ASTIKASARI[1], DITA RAHMAYANI[1], MUHAMMAD FADHILLAH ARMANDO[1], GILANG DWI NUGROHO[2], WIDHI HIMAWAN[1], ASEP ZAINAL MUTAQIN[3], DARLINA MD. NAIM[4], AHMAD DWI SETYAWAN[1,5,♥]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia. Tel./Fax.: +62-271-663375, ♥email: volatileoils@gmail.com
[2]Biodiversity Research Club, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia
[3]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. Jl. Raya Bandung-Sumedang Km 21, Jatinangor, Sumedang 45363, West Java, Indonesia
[4]School of Biological Sciences, Universiti Sains Malaysia. 1112, Persiaran Sains, 11800 Gelugor, Pulau Pinang, Malaysia
[5]Biodiversity Research Group, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia

Manuscript received: 6 January 2023. Revision accepted: 4 April 2023.

Abstract. *Sagitarian DGF, Astikasari L, Rahmayani D, Armando MF, Nugroho GD, Himawan W, Mutaqin AZ, Md. Naim D, Setyawan AD. 2023. Biodiversity of edible fruit sold at Pasar Gede, Surakarta City, Central Java, Indonesia. Asian J Agric 7: 57-68.* The market is a place to purchase numerous varieties of goods. Pasar Gede is a traditional Indonesian marketplace in Surakarta City, Central Java, Indonesia. It is the earliest and the largest fruit market in Surakarta. This study was conducted to ascertain the variety species of fruits sold at Pasar Gede from 12 to 16 December 2022. Direct interviews with Pasar Gede administrators and sellers conducted the sampling. This study uses primary data from interviews and secondary data from literature studies to support the primary data, while descriptive analysis was performed on the collected data. The results revealed that the fruit sold at Pasar Gede comprised 82 species from 25 families, with information on local name, price, seasonality, rarity according to vendors, and conservation status according to the IUCN Red List. In Pasar Gede, fruits with conservation status Least Concern (LC) based on the IUCN Red List were found, namely *juwet* (*Syzygium cumini* (L.) Skeels), *matoa* (*Pometia pinnata* J.R.Forst. & G.Forst.), *jambu biji* (*Psidium guajava* L.), *pir packham* (*Pyrus communis* L.), *srikaya* (*Annona squamosa* L.), *jeruk bali* (*Citrus maxima* (Burm.) Merr.), *delima arab* (*Punica granatum* L.), *sirsak* (*Annona muricata* L.), *sawo* (*Manilkara zapota* (L.) P.Royen), *asam jawa* (*Tamarindus indica* L.) and *kesemek* (*Diospyros kaki* L.f.). *Jeruk* (*Citrus* sp.), *apel/pir* (*Pyrus* sp.), *pisang* (*Musa* sp.), *mangga* (*Mangifera* sp.), and *alpukat* (*Persea* sp.) are the dominant fruits (the most available and frequently sold by every vendor in his stall) in Pasar Gede. *Anggur autum* (*Vitis vinifera* L. cv. autumn) are the most expensive products at 180,000 IDR per kilogram, while *jambu biji* is the least expensive at 8,000 IDR per kilogram. The production cost, seasonality, and fruit quality determine whether fruit prices are costly. The abundance of fruit indicates that Pasar Gede is a comprehensive fruit market center in Indonesia, particularly in Surakarta.

Keywords: Import, local fruit, rare, traditional market

INTRODUCTION

The fruit is part of the plant, which consists of seeds and the outer part of the fruit/epicarp (Esfahlan et al. 2019). Fruit is a source of minerals, carotene, and energy for the human body (Dhok et al. 2020). The fruit has broad benefits for humans, such as being consumed as food tenacity and functioning in medicine (Suwardi et al. 2020). Every human being needs consumption, especially fruit (Apriliani et al. 2021), which causes the demand continuously. Consuming proper fruit every day is essential for health. Still, according to Komarayanti (2017), the Indonesian population consumes less fruit and has not yet reached a sufficient level (minimum 150 grams/capita/day), which is recommended by WHO (Indonesian Ministry of Agriculture 2021). According to BPS Indonesia (2021), Indonesian consume fruit only around 88.56 grams/capita/day of species of fruit such as *jeruk* (*Citrus* sp.), *rambutan* (*Nephelium* sp.), *duku* (*Lansium* sp.), *durian* (*Durio* sp.), *apel/pir* (*Pyrus* sp.), *salak* (*Salacca* sp.), *pisang* (*Musa* sp.), and *pepaya* (*Carica* sp.). These amounts are still below the minimum limit recommended by WHO. Indonesia is a country that has a wide variety of fruit species, recording 592 species, with 22 commercial fruit (Kumoro et al. 2020). Several native fruits in Indonesia have high economic value and species diversity, such as *mangga* (*Mangifera* sp.), *durian* (*Durio* sp.), and *rambutan* (*Nephelium* sp.) (Angio and Irawanto 2019). Other fruits that can be easily found in Indonesia are *apel/pir* (*Pyrus* sp.) and *jeruk* (*Citrus* sp.). Almost all species of *apel/pir* (*Pyrus* sp.) are consumed directly or, after processing, as chips or drinks (Safitri et al. 2019). *Jeruk* (*Citrus* sp.) is also a fruit widely consumed by Indonesians and is a superior commodity because of its high consumption rate and ease of cultivation (Astuthi and Antasari 2020).

Several species of local fruit are not widely known and have not been documented (Noverian et al. 2020). Hence, the conservation and sustainable use of these fruit varieties

and the diversity of their consumption are not optimal (Harris et al. 2022). Several factors also influence this condition, including the species of fruit (seasonal or not), land conversion, and imported fruit into the local market (Pratama et al. 2019). Intensive land conversion factors can also cause a loss of fruit biodiversity (Horak et al. 2013). Differences in geographical conditions in each region can influence the diversity of fruit species, thus creating fruit species with characteristics according to their origin (Priyambodo et al. 2019). In addition, the diversity of fruit species can increase because new varieties are created from gene-crossing technology per market demand or other methods (Fitriani et al. 2014). However, the presence of environmental factors such as temperature rise will affect the condition of the fruit. An increase in temperature can trigger changes in taste. It occurs in *anggur* (*Vitis* sp.) and *alpukat* (*Persea* sp.) (Leisner 2020). Therefore, it will affect the quality and quantity of fruit sold. Although the diversity of fruit species in Indonesia is high, the season strongly influences fruit availability.

Traditional markets remain influential outlets for fruit sales (Jun et al. 2022). Traditional markets are physically accessible to low-income consumers and consumers who function as wholesalers, serve as entry points for small farmers, and provide job opportunities for sellers (Davies et al. 2022). The fruit market in Indonesia has received a lot of imported fruit inputs. This expansion is because the quality of local fruit has not been able to create superior products (Rahayu et al. 2012). One of the markets with the main selling commodity for fruit in Indonesia is Pasar Gede, one of the leading markets in Surakarta City (Asyfiradayati et al. 2018), a cultural heritage market has attractiveness and competitiveness (Aliyah et al. 2017). In addition, Pasar Gede potentially attracts buyers from outside and within the city due to its strategic middle city location (Aliyah and Aulia 2019). From time to time, the main commodity sold at Pasar Gede varies from local and imported fruits. None of the various species of fruit sold at Pasar Gede come from Surakarta City, but stocks come from Salatiga, Karanganyar, Boyolali, Madiun, and outside Java Island. It is influenced by the condition of Surakarta's land, an urban area that has increased rapidly and resulted in a loss of agricultural land and fruit cultivation (Rada et al. 2022). Based on the preliminary survey, while Pasar Gede is the largest fruit market in Surakarta, no research discusses the diversity of fruit sold there. Hence, this study aimed to determine the diversity of fruits sold in Pasar Gede, Surakarta, Central Java, Indonesia.

MATERIALS AND METHODS

Study area

This research was conducted on 12-16 December 2022 at Pasar Gede Hardjonagoro (traditional market), commonly called Pasar Gede. Pasar Gede is located on Jebres Sub-district, Surakarta City, Central Java, Indonesia, with coordinates 7°34'07" S, 110°49'52" E (Figure 1). Pasar Gede is a fruit market in Surakarta, built from 1925 to 1930 (Herlambang et al. 2017). Based on information from Agus Suharto as the Market Management, it is the largest fruit market in Surakarta. Then, Pasar Gede was a cultural heritage market officially operated in 1935 (Figure 2). Pasar Gede has the full name Pasar Gede Hardjonagoro, derived from the name of a Chinese nobleman who received the title Kanjeng Raden Tumenggung Hardjonagoro. This Chinese nobleman was the one who started Pasar Gede in the form of a *plataran*. In Surakarta, this market is the oldest, and during the golden age of the Surakarta Hadiningrat Palace, it played a role as the center of the economy. In the context of developing Surakarta's economy, Pasar Gede was built and designed by Ir. Herman Thomas Karsten during the time of Pakubuwono X. The Pasar Gede building, consisting of 2 floors, has a unique shape. It is an old Javanese colonial architecture that has maintained its shape until now (Soebiyan et al. 2020). The distinctive shape of the building makes it different from most markets in Surakarta, so it has its charm. Pasar Gede is a traditional Javanese model market that provides staple goods for people outside the palace, such as Chinese Ethnicity, Indigenous Javanese, and Dutch (Harsasto 2018).

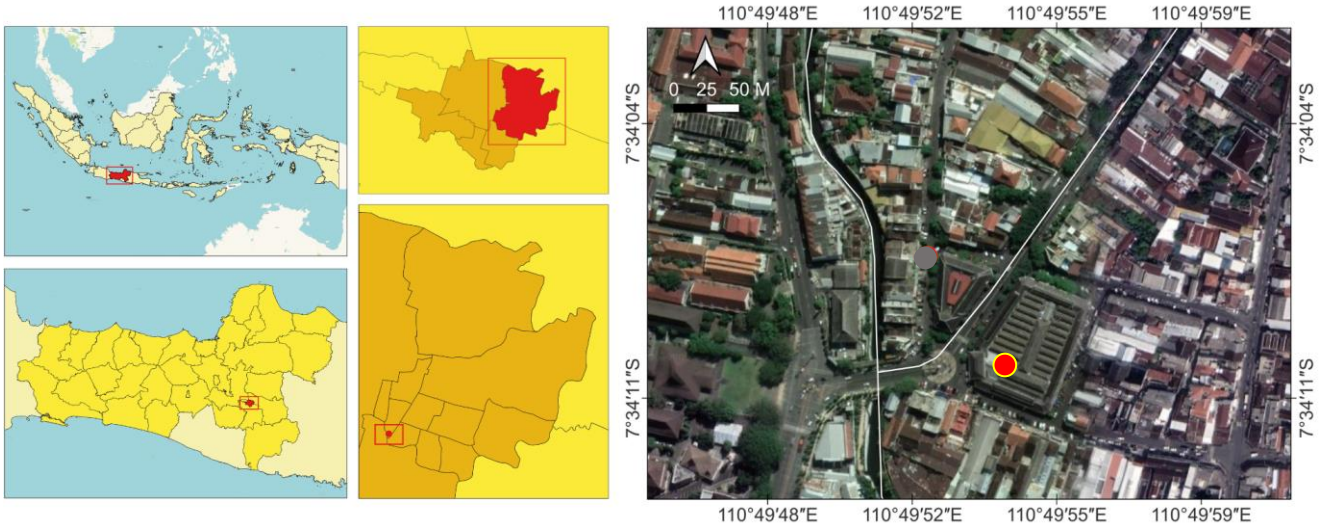


Figure 1. Location of Pasar Gede in Sudiroprajan Village, Jebres Sub-district, Surakarta City, Central Java, Indonesia

Figure 2. Pasar Gede building, Surakarta, Central Java, Indonesia in 1935 (*left*) and 2022 (*right*)

Agus Suharto further informed that with the development of times, this market has also begun to develop in providing various needs for the community. Even so, the goods traded during construction are still the same: necessities (rice, sugar, oil, eggs, onions, salt, meat, milk, kerosene and Liquid Petroleum Gas), vegetables, fish, and pork in the form of meat or processed. For now, there is extra fruit sold in it. As a result of the constantly high demand for fruit, the supply at Pasar Gede is of various species, ranging from local to imported fruits. It resulted in Pasar Gede becoming the most significant fruit market in Surakarta City. Compared to other traditional market, its presence is the oldest and the center of the community's economy and culture. Cultural diversity can enhance traditional culture through various cultural events that attract national and international attention (Ekomadyo 2019).

Data collection procedures

The data source of this research is divided into primary and secondary data. Primary data was obtained by conducting interviews with all fruit sellers in Pasar Gede (a total of 48 sellers) to observe whether fruits were sold, local names, prices, scarcity according to the sellers, and seasonal fruit. Interviews with sellers were conducted through a prepared questionnaire (Ruwaida et al. 2022). Meanwhile, secondary data was obtained from literature studies to support data in the field. Secondary data in this study was obtained by family groups, Latin names, and conservation status (IUCN). Various species of fruit sold by sellers in Pasar Gede were recorded in the questionnaire and documented.

Data analysis

Data analysis was carried out descriptively and supported by tables, pictures, and graphs as explanations. Tables are presented to explain the characteristics of the respondents and the variety of fruit found in Pasar Gede. Graphs are provided to analyze the family of each fruit found. Apart from being in tabular form, the variety of fruits is also presented in documentation or pictures.

RESULTS AND DISCUSSION

Characteristics of respondents from Pasar Gede fruit sellers

The characteristics of fruit sellers in Pasar Gede, based on the interviews with all fruit sellers, are shown in Table 1. This table shows that most fruit sellers in Pasar Gede are women (39), while only nine are men. The most common age of sellers ranges from 51-60 years, while most sellers had high school or elementary school backgrounds. The seller's age can affect their experience, so the seller better understands consumer desires. Several fruit vendors have been selling at Pasar Gede for decades, several respondents even stated that selling fruit at Pasar Gede was a business passed down from their parents. It makes sellers already understand the characteristics of consumers who come. Therefore, sellers are becoming more focused on selecting fruit consumers' requests. On the contrary, being too old will make it difficult for sellers to trade because their increasingly weak physical condition limits their movements. A high educational background shows an individual's knowledge, so sellers with a higher educational background should be more competent in managing their business. However, it is not always accurate because trading requires knowledge and skills. Therefore, good educational background, adequate trading skills, and experience are indispensable for sellers. At Pasar Gede, vendors sell many species of local and imported fruit; some sellers have been selling for over 20 years, and selling fruits was their parents' job. Meanwhile, there was also an elderly merchant who had only graduated from elementary school. She sells only 1 species of fruit, which is usually local fruit and grown by herself.

Fruit family

Based on Figure 3, it can be seen that there are 25 families of fruit found in Pasar Gede. Most families are represented by only one species: Cactaceae, Oxalidaceae, Bromeliaceae, Chenopodiaceae Actinidiaceae, Lythraceae, Sapotaceae, Fabaceae, Bombacaceae, Clusiaceae, Meliaceae, and Ebenaceae. The fruit family with the most species for sale is Rosaceae, with 15 species; Rutaceae,

with 11 species; and Anacardiaceae and Musaceae, with nine species. This diagram shows that the diversity of families of fruit sold at Pasar Gede is quite diverse compared to those found at Pasar Legi Surakarta, where only 16 families were found for fruits sold at the market (Nurshillah et al. 2022). However, an overview of the species diversity of each family is still lacking because most families only contribute to one species.

Variety species of fruit in Pasar Gede

Based on the interviews with sellers, information was obtained that sellers sold as many as 82 species of fruit in stalls or kiosks (Table 2). These fruits are local Indonesian fruits and imported fruits. As much as 75% (36 sellers) mostly sell citrus fruits or *jeruk* (*Citrus* spp.), apples or *apel/pir* (*Pyrus* spp.), bananas/*pisang* (*Musa* spp.), mangoes/*mangga* (*Mangifera* spp.) and avocados/*alpukat* (*Persea* spp.). Then, the remaining 25% (12 sellers) are sellers who sell species of fruit that other sellers rarely sell, such as (i) local fruit, namely persimmon/*kesemek* (*D. kaki*), *matoa* (*P. pinnata*), *siwalan* (*B. flabellifer*), *srikaya* (*A. squamosa*), *kedondong* (*S. dulcis*), *sukun* (*A. altilis*), *blewah* (*C. melo* var. cantalupensis naudin), *asam jawa* (*T. indica*), and *juwet* (*S. cumini*); (ii) imported fruit, namely plums/*plum* (*P. domestica*), *bit* (*B. vulgaris*), lychees/*leci* (*L. chinensis*), and Arabic pomegranates/*delima arab* (*P. granatum*). Some pictures of fruit sold at Pasar Gede are shown in Figure 4.

Rarity and conservation status

The availability of fruit is seasonal and year-round, with almost the same amount. However, the availability of 5 species of fruit is rare, 4 seasonal fruits (*B. flabellifer, S. cumini, P. domestica,* and *P. pinnata*) and 1 year-round fruit (*B. vulgaris*). The fruit with the highest selling price is the autumn grape/*anggur autum* (*V. vinifera* var. autumn), with a price of 180,000 IDR per kilogram, which is imported fruit. Then, the fruit with the lowest selling price is *P. guajava* at 8,000 IDR per kilogram. Table 2 shows the fruit species with the Least Concern (LC) conservation status (low risk) based on the IUCN Red List are *S. cumini*, *P. pinnata*, *P. guajava*, *P. communis*, *A. squamosa*, *C. maxima*, *P. granatum*, *A. muricata*, *M. zapota*, *T. indica*, and *D. kaki*. Table 3 also shows that the fruit species sold at Pasar Gede are more diverse and in greater quantity than in other markets. Several species of fruit at Pasar Gede, Surakarta, Indonesia can be seen in Figure 5.

Table 1. Characteristics of respondents from fruit sellers at Pasar Gede, Surakarta, Central Java, Indonesia (n= 48)

Variable	Amount	Percentage (%)
Age		
20-30	2	4.2
31-40	4	8.3
41-50	13	27.1
51-60	17	35.4
61-70	9	18.7
71-80	2	4.2
81-90	1	2.1
Gender		
Male	9	18.75
Female	39	81.25
Education		
Elementary School	24	50.00
Junior High School	9	18.75
Senior High School	15	31.25
University	0	0

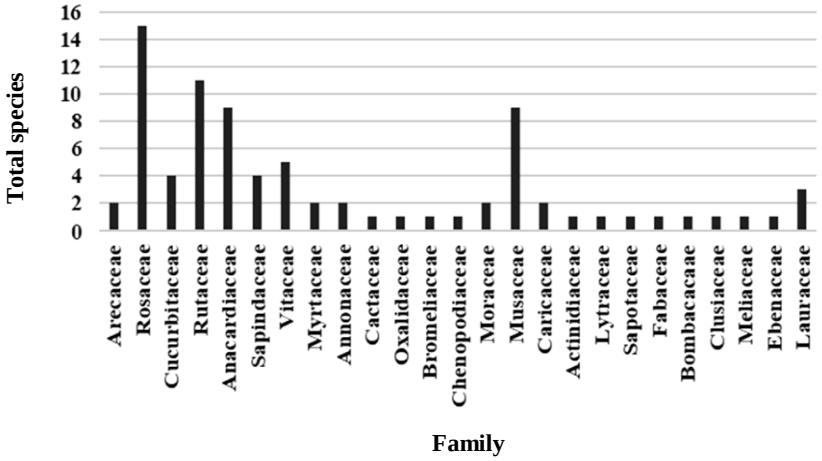


Figure 3. The number of species in the fruit family sold at Pasar Gede, Surakarta, Central Java, Indonesia

A **B** **C**

Figure 4. General conditions inside the market (A and B) and vendor stalls (C) in Pasar Gede, Surakarta, Central Java, Indonesia

Table 2. The variety of fruit sold at Pasar Gede, Surakarta, Central Java, Indonesia

Family	Scientific name	Local name	Price (IDR)	Fruit category (Seasonal/ Year-round)	Information (Rare/ Not-Rare)	Status IUCN
Actinidiaceae	*Actinidia deliciosa* (Chev.) C.F.Liang & A.R.Ferguson	*Kiwi*	60,000/kg	Seasonal	Not Rare	-
Anacardiaceae	*Mangifera indica* L. cv. arummanis	*Mangga arumanis*	15,000/kg	Seasonal	Not Rare	-
Anacardiaceae	*Mangifera indica* L. cv. gadung	*Mangga gadung*	25,000/kg	Seasonal	Not Rare	-
Anacardiaceae	*Mangifera indica* L. cv. gedong gincu	*Mangga gedong gincu*	55,000/kg	Seasonal	Not Rare	-
Anacardiaceae	*Mangifera indica* L. cv. indramayu	*Mangga indramayu*	30,000/kg	Seasonal	Not Rare	-
Anacardiaceae	*Mangifera indica* L. cv. manalagi	*Mangga manalagi*	20,000/kg	Seasonal	Not Rare	-
Anacardiaceae	*Mangifera indica* L. cv. okyong	*Mangga okyong*	40,000/kg	Seasonal	Not Rare	-
Anacardiaceae	*Mangifera indica* L. cv. alpukat	*Mangga alpukat*	35,000/kg	Seasonal	Not Rare	-
Anacardiaceae	*Mangifera lalijiwa* Kosterm.	*Mangga lalijiwo*	15,000/kg	Seasonal	Not Rare	-
Anacardiaceae	*Spondias dulcis* Sol. ex G.Forst.	*Kedondong*	25,000/kg	Year-round	Not Rare	-
Annonaceae	*Annona muricata* L.	*Sirsak*	20,000/kg	Year-round	Not Rare	LC
Annonaceae	*Annona squamosa* L.	*Srikaya*	25,000/ kg	Year-round	Not Rare	LC
Arecaceae	*Borassus flabellifer* L.	*Siwalan*	15,000/pcs	Seasonal	Rare	-
Arecaceae	*Salacca zalacca* (Gaertn.) Voss cv. pondoh	*Salak pondoh*	25,000/kg	Year-round	Not Rare	-
Bombacacaae	*Durio zibethinus* Murray	*Durian*	50,000/kg	Seasonal	Not Rare	-
Bromeliaceae	*Ananas comosus* (L) Merr.	*Nanas madu*	15,000/pcs	Year-round	Not Rare	-
Cactaceae	*Selenicereus undatus* (Haw.) D.R.Hunt	*Buah naga merah*	15,000/kg	Year-round	Not Rare	-
Caricaceae	*Carica papaya* L. cv. california	*Pepaya california*	10,000/ kg	Year-round	Not Rare	-
Caricaceae	*Carica papaya* L. cv. thailand	*Pepaya thailand*	15,000/kg	Year-round	Not Rare	-
Chenopodiaceae	*Beta vulgaris* L.	*Buah bit*	25,000/kg	Year-round	Rare	-
Clusiaceae	*Garcinia mangostana* L.	*Manggis*	40,000/kg	Seasonal	Not Rare	-
Cucurbitaceae	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	*Semangka*	10,000/kg	Year-round	Not Rare	-
Cucurbitaceae	*Cucumis melo* L.	*Melon*	20,000/kg	Seasonal	Not Rare	-
Cucurbitaceae	*Cucumis melo* L. cv. cantalupensis Naudin	*Blewah*	20,000/kg	Seasonal	Not Rare	-
Cucurbitaceae	*Cucumis melo* L. cv. golden	*Melon golden*	40,000/kg	Seasonal	Not Rare	-
Ebenaceae	*Diospyros kaki* L.f.	*Kesemek*	30,000/kg	Seasonal	Not Rare	LC
Fabaceae	*Tamarindus indica* L.	*Asam jawa*	25,000/kg	Year-round	Not Rare	LC
Lauraceae	*Persea americana* Mill.	*Alpukat mentega*	25,000/kg	Seasonal	Not Rare	-
Lauraceae	*Persea americana* Mill. cv. aligator	*Alpukat aligator*	30,000/kg	Seasonal	Not Rare	-
Lauraceae	*Persea americana* Mill. cv. kendil	*Alpukat kendil*	40,000/kg	Seasonal	Not Rare	-
Lytraceae	*Punica Granatum* L.	*Delima arab*	35,0000/kg	Year-round	Not Rare	LC
Meliaceae	*Lansium domesticum* Corrêa	*Duku*	45,000/kg	Seasonal	Not Rare	-
Moraceae	*Artocarpus altilis* (Parkinson) Fosberg	*Sukun*	15,000/kg	Seasonal	Not Rare	-
Moraceae	*Artocarpus heterophyllus* Lam.	*Nangka*	40,000/kg	Year-round	Not Rare	-
Musaceae	*Musa acuminata* Colla cv. AA	*Pisang barlin*	15,000/comb	Year-round	Not Rare	-
Musaceae	*Musa acuminata* Colla cv. cavendish	Pisang cavendish	30,000/comb	Year-round	Not Rare	-
Musaceae	*Musa acuminata* Colla cv. raja	*Pisang raja*	30,000/comb	Year-round	Not Rare	-
Musaceae	*Musa acuminata* Colla cv. susu	*Pisang susu*	15,000/comb	Year-round	Not Rare	-
Musaceae	*Musa acuminata* Colla cv. susu merah	*Pisang susu merah*	40,000/comb	Year-round	Not Rare	-
Musaceae	*Musa acuminata x M. balbisiana*	*Pisang kepok*	30,000/comb	Year-round	Not Rare	-

Musaceae	*Musa paradisiaca* L. cv. bawen	*Pisang bawen*	20,000/comb	Year-round	Not Rare	-
Musaceae	*Musa paradisiaca* L. cv. mas	*Pisang mas*	25,000/comb	Year-round	Not Rare	-
Musaceae	*Musa paradisiaca* L. cv. sapientum	*Pisang ambon*	50,000/comb	Year-round	Not Rare	-
Myrtaceae	*Psidium guajava* L.	*Jambu biji*	8,000/kg	Year-round	Not Rare	LC
Myrtaceae	*Syzygium cumini* (L.) Skeels	*Juwet*	110,000/ kg	Seasonal	Rare	LC
Oxalidaceae	*Averrhoa carambola* L. cv. demak	*Belimbing demak*	25,000/kg	Year-round	Not Rare	-
Rosaceae	*Fragaria* × *ananassa* (Weston) Rozier	*Stroberi*	60,000/kg	Year-round	Not Rare	-
Rosaceae	*Malus domestica* (Suckow) Borkh.	*Apel hijau*	25,000/kg	Year-round	Not Rare	-
Rosaceae	*Malus domestica* (Suckow) Borkh. cv. Ambrosia	*Apel ambrosia*	90,000/kg	Year-round	Not Rare	-
Rosaceae	*Malus domestica* (Suckow) Borkh. cv. granny smith	*Apel granny smith*	60,000/kg	Year-round	Not Rare	-
Rosaceae	*Prunus domestica* L.	*Plum*	30,000/kg	Seasonal	Rare	-
Rosaceae	*Pyrus communis* L.	*Pir packham*	50,000/kg	Seasonal	Not Rare	LC
Rosaceae	*Pyrus communis* L. cv. century	*Pir century*	25,000/kg	Seasonal	Not Rare	-
Rosaceae	*Pyrus communis* L. cv. yali	*Pir yali*	45,000/kg	Seasonal	Not Rare	-
Rosaceae	*Pyrus malus* L.	*Apel*	35,000/kg	Year-round	Not Rare	-
Rosaceae	*Pyrus malus* L. cv. fuji	*Apel fuji*	35,000/kg	Year-round	Not Rare	-
Rosaceae	*Pyrus malus* L. cv. madu	*Pir madu*	25,000/kg	Seasonal	Not Rare	-
Rosaceae	*Pyrus malus* L. cv. washington	*Apel washington*	40,000/kg	Year-round	Not Rare	-
Rosaceae	*Pyrus pyrifolia* (Burm.fil.) Nakai	*Pir asia*	50,000/kg	Seasonal	Not Rare	-
Rosaceae	*Pyrus pyrifolia* (Burm.fil.) Nakai cv. singo	*Pir korea singo*	80,000/kg	Seasonal	Not Rare	-
Rosaceae	*Pyrus pyrifolia* (Burm.fil.) Nakai cv. xiang lie	*Pir xiang lie*	30,000/kg	Seasonal	Not Rare	-
Rutaceae	*Citrus* × *aurantiifolia* (Christm.) Swingle	*Jeruk nipis*	20,000/kg	Year-round	Not Rare	-
Rutaceae	*Citrus limon* (L.) Osbeck	*Lemon*	30,000/kg	Year-round	Not Rare	-
Rutaceae	*Citrus maxima* (Burm.) Merr.	*Jeruk bali*	25,000/kg	Year-round	Not Rare	LC
Rutaceae	*Citrus nobilis* Andrews	*Jeruk medan*	25,000/kg	Year-round	Not Rare	-
Rutaceae	*Citrus reticulata* Blanco	*Jeruk mandarin*	30,000/kg	Year-round	Not Rare	-
Rutaceae	*Citrus reticulata* Blanco cv. shiranui	*Jeruk dekopon*	90,000/kg	Year-round	Not Rare	-
Rutaceae	*Citrus sinensis* (L) Osbeck cv. sunkist	*Jeruk sunkist*	40,000/kg	Year-round	Not Rare	-
Rutaceae	*Citrus sinensis* (Mill.) Pers. cv. baby	*Jeruk baby*	15,000/kg	Year-round	Not Rare	-
Rutaceae	*Citrus sinensis* L. Osbeck cv. manis	*Jeruk manis*	25,000/kg	Year-round	Not Rare	-
Rutaceae	*Citrus* sp.	*Jeruk santang*	40,000/kg	Year-round	Not Rare	-
Rutaceae	*Triphasia trifolia* (Burm.fil.) P.Wilson	*Jeruk kimkit*	100,000/kg	Year-round	Not Rare	-
Sapindaceae	*Dimocarpus longan* Lour.	*Kelengkeng*	40,000/kg	Year-round	Not Rare	-
Sapindaceae	*Litchi chinensis* Sonn.	*Leci*	140,000/kg	Seasonal	Not Rare	-
Sapotaceae	*Manilkara zapota* (L.) P.Royen	*Sawo*	20,000/kg	Seasonal	Not Rare	LC
Sapindaceae	*Nephelium lappaceum* L.	*Rambutan*	30,000/kg	Seasonal	Not Rare	-
Sapindaceae	*Pometia pinnata* J.R.Forst. & G.Forst.	*Matoa*	50,000/kg	Seasonal	Rare	LC
Vitaceae	*Vitis vinifera* L. cv. australia	*Anggur australia*	50,000/kg	Year-round	Not Rare	-
Vitaceae	*Vitis vinifera* L. cv. autumn	*Anggur autum*	180,000/kg	Year-round	Not Rare	-
Vitaceae	*Vitis vinifera* L. cv. muscat	*Anggur muscat*	140,000/kg	Year-round	Not Rare	-
Vitaceae	*Vitis vinifera* L. cv. red	*Anggur merah*	50,000/kg	Year-round	Not Rare	-
Vitaceae	*Vitis vinifera* L. cv. RRC	*Anggur RRC*	50,000/kg	Year-round	Not Rare	-

Note: LC: Least Concern based on the IUCN Red List, IDR: Indonesian Rupiah

Table 3. Several traditional markets in Indonesia and abroad

Traditional market names	Number of species of fruit	References
Ujung Berung traditional market, Bandung (Indonesia)	39	Iskandar et al. (2018)
Sukoharjo traditional market (Indonesia)	32	Deanova et al. (2021)
Beringharjo traditional market, Yogyakarta (Indonesia)	55	Iskandar et al. (2021)
Tabanan traditional market, Bali (Indonesia)	9	Sujarwo et al. (2018)
Karawang village traditional market, Cianjur (Indonesia)	8	Iskandar et al. (2020)
Legi market, Surakarta (Indonesia)	24	Nurshillah et al. (2022)
Traditional market in Bekasi (Indonesia)	24	Gordi et al. (2022)
Tamu Kianggeh Market (Brunei Darussalam)	31	Franco et al. (2020)
Fergana Valley traditional market (Southern Kyrgyzstan)	7	Vlkova et al. (2015)
Batu Pahat traditional market (Malaysia)	22	Sulaini and Sabran (2018)


1


2


3


4


5


6


7


8


9


10


11


12


13


14


15


16


17


18


19


20


21


22


23


24


25


26


27


28


29


30


31


32


33


34


35


36


37


38


39


40

41

42

43

44

45

46

47

48

Figure 5. Several species of fruit at Pasar Gede, Surakarta, Indonesia. 1. *Kiwi* (*Actinidia deliciosa*), 2. *Mangga indramayu* (*Mangifera indica* cv. indramayu), 3. *Mangga gedong gincu* (*Mangifera indica* cv. gedong gincu), 4. *Srikaya* (*Annona squamosa*), 5. *Sirsak* (*Annona muricata*), 6. *Salak pondoh* (*Salacca zalacca* cv. pondoh), 7. *Nanas madu* (*Ananas comosus*), 8. *Buah naga merah* (*Selenicereus undatus*), 9. *Pepaya california* (*Carica papaya* cv. california), 10. *Pepaya thailand* (*Carica papaya* cv. thailand), 11. *Bit* (*Beta vulgaris*), 12. *Semangka* (*Citrullus lanatus*), 13. *Melon* (*Cucumis melo*), 14. *Melon golden* (*Cucumis melo* cv. golden), 15. *Blewah* (*Cucumis melo* cv. cantalupensis), 16. *Pisang ambon* (*Musa paradisiaca* cv. sapientum), 17. *Pisang cavendish* (*Musa acuminata* cv. cavendish), 18. *Alpukat aligator* (*Persea americana* cv. aligator), 19. *Alpukat kendil* (*Persea americana* cv. kendil), 20. *Alpukat mentega* (*Persea americana*), 21. *Anggur merah* (*Vitis vinifera* cv. red), 22. *Apel hijau* (*Malus domestica* cv. Ambrosia), 23. *Apel fuji* (*Pyrus malus* cv. fuji), 24. *Nangka* (*Artocarpus heterophyllus*), 25. *Pisang bawen* (*Musa paradisiaca* cv. bawen), 26. *apel* (*Pyrus malus*), 27. *Apel washington* (*Pyrus malus* cv. washington), 28. *Belimbing demak* (*Averrhoa carambola* cv. demak), 29. *Asam jawa* (*Tamarindus indica*), 30. *Delima arab* (*Punica granatum*), 31. *Mangga gadung* (*Mangifera indica* cv. gadung), 32. *Pir asia* (*Pyrus pyrifolia*), 33. *Mangga arumanis* (*Mangifera indica* cv. arummanis), 34. *jambu biji* (*Psidium guajava*), 35. *Jeruk bali* (*Citrus maxima*), 36. *Jeruk baby* (*Citrus sinensis* cv. baby) 37. *Jeruk dekopon* (*Citrus reticulata* cv. shiranui), 38. *Jeruk mandarin* (*Citrus reticulata*), 39. *Jeruk manis* (*Citrus sinensis* cv. manis), 40. *Jeruk nipis* (*Citrus* × *aurantiifolia*), 41. *Jeruk sunkist* (*Citrus sinensis* cv. sunkist), 42. *Kelengkeng* (*Dimocarpus longan*), 43. Lemon (*Citrus limon*), 44. *Mangga lalijiwo* (*Mangifera lalijiwa*), 45. *Pir yali* (*Pyrus communis* cv. communis), 46. *pir packham* (*Pyrus communis*), 47. *Pir korea singo* (*Pyrus pyrifolia* cv. singo), 48. *Pir xiang lie* (*Pyrus pyrifolia* cv. xiang lie), 49. *Pisang barlin* (*Musa acuminata* AA), 50. *Pisang kepok* (*Musa acuminata x M. balbisiana*), 51. *Pisang susu merah* (*Musa acuminata* cv. Susu merah), 52. *Plum* (*Prunus domestica*), 53. *Rambutan* (*Nephelium lappaceum*), 54. *Jeruk santang* (*Citrus* sp.), 55. *Sawo* (*Manilkara zapota*), 56. Stroberi (*Fragaria* ×*ananassa*), 57. *Sukun* (*Artocarpus altilis*), 58. *Anggur asutralia* (*Vitis vinifera* cv. australia), 59. *Anggur autum* (*Vitis vinifera* cv. Autumn), 60. *Juwet* (*Syzygium cumini*), 61. Anggur muscat (*Vitis vinifera* cv. muscat), 62. *Jeruk kimkit* (*Triphasia trifolia*), 63. *Matoa* (*Pometia pinnata*), 64. *Kedondong* (*Spondias dulcis*), 65. *Mangga okyong* (*Mangifera indica* cv. okyong), 66. *Apel granny smith* (*Malus domestica* cv. granny smith)

Discussions

In this study, 82 species of fruit were sold at Pasar Gede, both local and imported. Table 3 shows that Pasar Gede has more fruit sold than other markets from several traditional markets in Indonesia and abroad. Buyers can buy daily needs at this market because of its location (in the middle of the city), complete goods, and best quality goods at affordable prices (Puteri and Fajarwati 2016). The interview with Mr. Agus Suharto (manager of Pasar Gede) shows that the sellers strive for the best quality and maintain the sustainability of the fruit commodity sold by supplying it from out of town and abroad. Furthermore, Mr. Agus Suharto informs that the large number of buyers at Pasar Gede has an impact on increasing demand for fruit so that the species of fruit sold could be more diverse. The fruits buyers at Pasar Gede seek more after are citrus (*Citrus* spp.) because the community widely uses it for important events, such as the Chinese New Year.

The impact of imports from abroad has resulted in the price of fruit at Pasar Gede tending to be more expensive than at other traditional markets in Surakarta. However, it is still affordable for the community. Fruit sold at higher prices at Pasar Gede is also due to their type as seasonal fruits such as *S. cumini*, *P. pinnata*, *M. indica* cv. *gedong gincu*, *D. zibethinus*, *L. domesticum*, *L. chinensis*, *D. kaki*, *N. lappaceum*, *A. deliciosa*, *G. mangostana*, several species of avocado/*alpukat*, namely *kendil* (*P. americana* cv. *kendil*) and alligator (*P. americana* cv. *alligator*). Seasonal fruits found in Pasar Gede are generally expensive because they are not sold during their fruiting season, so they are

rarely found. When it sells during their fruiting season, the price will be cheaper; we could also find year-round fruits at a lower price than seasonal fruit. Autumn grapes (*V. vinifera* cv. autumn) are the fruit type with the highest price of 180,000 IDR/kg. Autumn grapes are seedless grapes usually served at banquets. This fruit has high qualities such as higher nutrition, larger diameter, and sweeter than other species because it is produced under strict management and various studies to produce this high-quality fruit (Faci et al. 2014; Fuentes et al. 2018). The overall costs during the fruit production period influence the fruit selling price (Panagos et al. 2018). High quality, being a cultivar, and having high production costs make the price of autumn grapes more expensive. In addition, citrus fruits dominate sales (36 sellers) because they have affordable and very abundant prices (Setiawan et al. 2019). On the contrary, the fruits that people rarely demand, such as *sukun* (*A. altilis*), although it is a seasonal fruit, the price is cheaper than other seasonal fruits.

Seasonal fruits are superior commodities for export and have high economic value. Due to influencing factors, seasonal fruit tends to be more difficult to produce and sustain. One of them is the biennial-bearing characteristic of some plants. Biennial bearing is inconsistent in the stages of fruiting and flowering. A tree will find abundant and maximum fruit yields when it bears fruit but will bear little fruit in the next fruiting time, resulting in erratic crop production (Shivran et al. 2020). The Biennial-bearing properties are caused by the young fruit, which produces the hormone gibberellin. This hormone is then distributed to the vegetative shoots resulting in dense leaves but no flowers in the following year resulting in no fruit (Widiatama et al. 2021). Moreover, seasonal fruit is also influenced by climates, such as rainfall, wind, and dry and wet months. It occurs in *durian* (*Durio* spp.), wherein the flowering and fruiting stages take about 1-2 dry periods/month to encourage the flowering process to bear fruit (Rudarmono et al. 2022). High rainfall will result in delays in the flowering process due to many flowers and young fruit falling, reducing the quantity of fruit. La Nina and El Nino can cause high and low rainfall intensity in this area. The existence of La Nina results in high rainfall, which can shed flowers. In contrast, El Nino results in low rainfall, which results in a drought so that flowers are unable to develop and produce fruit which can disrupt fruit production (Sarvina and Sari, 2017). Rainfall that affects the dry/wet months will affect the quality of seasonal fruit, such as the *rambutan* (*Nephelium* sp.), whose flesh will be thin if the dry season is too lengthy (Nugroho et al. 2019). In addition, the wind substantially impacts certain seasonal fruits, such as avocado (*Persea* spp.). In the fruit-producing stage, flowers must be pollinated by wind, so an adequate amount of wind is required. High wind velocities will result in broken branches and impede pollination. On the other hand, temperature and other environmental factors, such as humidity, can also affect a product's nutritional value and flavor (Leisner 2020). Similarly to avocados, the concentration of monounsaturated and polyunsaturated fatty acids increases with temperature (Pedreschi et al. 2016).

Many fruits sold at Pasar Gede are not native to Surakarta; some are brought from other cities and countries. Farmers from Tawangmangu, Karanganyar, Boyolali, Berastagi (Karo), Madiun City, Bali Island, Kalimantan Island, and the Riau Islands sell produce to Pasar Gede's fruit merchants. For example, the stock fruit for *juwet* (*S. cumini*), which is hard to find and only sold by two sellers, comes from Karanganyar District, and *C. reticulata* are sent from Tawangmangu District. In addition to *P. communis* cv. *yali* from China, *V. vinifera* cv. *autumn* from Australia, *C. reticulata* cv. *shiranui* from Japan, and *P. malus* cv. *washington* from the United States.

The fruit utilization for fresh consumption has increased annually; it tends to increase fruit production (Kondo et al. 2020). According to Simchon et al. (2022), this necessitates the supply from other cities, even importation from other countries, to satisfy the need for sustainable food, rising demand, and environmental pressures. Moreover, due to increased urbanization causing changes in land use from previously vegetated areas to built-up land, Surakarta City lacks planting land for fruit cultivation. Consequently, sellers order fruit outside the city and in other countries (Putra et al. 2018). Therefore, to a limited extent, fruits grown in Surakarta cannot meet demand, which only provides a small quantity for personal consumption. On the other hand, due to Surakarta City's rapid growth, changes in land use have reduced the amount of vacant land (Fajar and Taryono 2022). Data from 2020 by Saputra (2021) showed that there had been a change in land use in the City of Surakarta, including vacant land for industry and warehousing, which increased by 1,045; Open space for buildings increased by 5.62; moorland into settlements increased 2.92; and dry land into offices increased by 0.05.

Changes in land use are also influenced by population growth (Agwu et al. 2020). It will cause the structural characteristics of various land use types and land use system types to vary significantly. Consequently, there is insufficient land accessible for farmers to plant fruit trees per regional conditions, despite fruit trees playing an essential role in maintaining the ecological environment and generating enormous economic benefits (Tamang et al. 2019). Urban agriculture contributes to food security and human well-being and has numerous environmental advantages. However, the land allocation has diminished significantly (Dobson et al. 2020). Similarly, the limited land in Surakarta City hinders the community's ability to obtain sufficient quantities of various varieties of fruit. Using fruiting trees as part of urban agriculture in urban landscape planning is a significant challenge (Kazemi et al. 2018). The development of agriculture to meet the fruit demand in urban areas requires planting fruit trees with minimal watering requirements.

Fruit can be used for various purposes, including consumption, as a medicinal constituent, religious rituals, etc. Rare fruit could be defined: as grown in a difficult location, as annual fruit, as fruit that is rarely cultivated, as fruit that grows wild in forest areas and riverbanks, as fruit that is rarely known to the general public, or as fruit that humans dislike due to its flavor (Yuliawati et al. 2016). A fruit's rarity is caused by many factors, including the plant's

slow reproduction rate, the challenging environment to flourish, and its limited distribution. For instance, *T. indica* does not grow well in damp soil and will not flower if grown in wet tropical regions (Agus et al. 2014). In contrast, *S. cumini* is in short supply because there are so few production centers and cultivation (Hesthiati et al. 2019). In addition, many land conversions that result in limited land for fruit cultivation, forest or orchard fires, or changes in environmental conditions are additional causes of fruit scarcity. Increased public interest in imported fruit and the abundance of engineered fruits may also contribute to the scarcity of local fruit. Furthermore, applying genetic engineering to fruit plants can lead to losing local fruit genetic information (Akhmadi and Sumarmiyati 2015). The increase in demand for fruit continues to outpace the increase in supply, making fruit increasingly rare.

Fruits thrive in Indonesia, and many varieties of fruit fall under the rare category, limiting their availability. Even though these rare fruits have great benefits, they are also used as a medicine besides being consumed fresh. Rare or uncommon fruits are typically restricted to a small region. Buyers need rare fruits rich in health benefits; therefore, the fruit sellers in Pasar Gede competed to get those rare fruit stocks. Sellers in Karanganyar District/other areas acquire *S. cumini* from farmers; this fruit is helpful as an anti-infection drug and treats diabetes. Customers wishing for exotic fruits will first order from a selected seller. Then, the seller will place orders and communicate with farmers to get the fruit they want/order. Therefore, the demand for rare fruit will be fulfilled at a higher price due to their special requirements. It's also possible for sellers to try on selling another previously unexploited fruit to acquire as rare stocks. In addition to enforcing laws against rare plants and cultivating rare fruit on a large scale, the government has endeavored to preserve rare fruit.

The IUCN Red List gives global data on all species' conservation status and extinction risk (Brooks et al. 2019). The IUCN Red List is accurate for all plants' conservation, planning, and prioritization actions (Kaky and Gilbert 2019). The IUCN Red List provides adequate demographic information regarding the conservation status of numerous extant organisms based on various criteria (Garner et al. 2020). According to the IUCN Red List, the conservation status is Not Evaluated, Data Deficient, Least Concern, Near Threatened, Vulnerable, Endangered, and Critically Endangered. Several fruits sold at Pasar Gede have a conservation status of LC or Least Concern, according to IUCN Red List records; These fruits are *S. cumini*, *P. pinnata*, *P. guajava*, *P. communis*, *A. squamosa*, *C. maxima*, *P. grenatum*, *A. muricata*, *M. zapota*, *T. indica*, and *D. kaki* (Table 2). Least Concern status indicates that a species is of low risk or has a limited range but is not included in a higher IUCN category. Even though most fruits sold at Pasar Gede do not threaten the environment, this issue still requires attention. In addition, fruits with LC status will not necessarily be able to survive continuously, even if it presently has a good conservation status. Therefore, when it comes to extensively consumed and the continued use of large quantities will result in fruit rarity. In contrast, the risk of fruit scarcity can increase for rarely consumed fruits, as few people are interested in them, and their market availability diminishes (Lestari 2014).

This study concludes that the fruit sold at Pasar Gede comprises 82 species from 25 families, with information on local name, price, seasonality, rarity, and conservation status based on the IUCN Red List. According to the IUCN Red List, Pasar Gede is home to fruits with a conservation status of LC (Least Concern), including *juwet* (*S. cumini*), *matoa* (*P. pinnata*), *jambu biji* (*P. guajava*), *pir packham* (*P. communis*), *srikaya* (*A. squamosa*), *jeruk bali* (*C. maxima*), *delima arab* (*P. granatum*), *sirsak* (*A. muricata*), *sawo* (*M.* zapota), *asam jawa* (*T. indica*), and *kesemek* (*D. kaki*). In Pasar Gede, *jeruk* (*Citrus* sp.), *apel/pir* (*Pyrus* sp.), *pisang* (*Musa* sp.), *mangga* (*Mangifera* sp.), and *alpukat* (*Persea* sp.) predominate (are the most available and frequently sold by every vendor in his kiosk). Autumn grapes (*V. vinifera* cv. *autumn*) are the most expensive products at 180,000 IDR per kilogram, while *jambu biji* is the least expensive at 8,000 IDR per kilogram. The cost of production, seasonality, and fruit quality determine the prices. The abundance of fruit indicates that Pasar Gede is a comprehensive fruit market center in Indonesia, particularly in Surakarta.

ACKNOWLEDGEMENTS

Researchers would like to express gratitude and appreciation to all parties which inspired, guided, and supported them in preparing this article. In particular, the researcher would like to thank Agus Suharto, the manager of Pasar Gede and all of the merchants at Pasar Gede, Surakarta, Central Java, Indonesia, for their willingness to serve as research subjects and resource persons for this article's discussion.

REFERENCES

Agus C, Adriyanti DT, Syahbudin A, Sargana D, Basori AF. 2014. Tanaman Langka Indonesia: di KP4 UGM. Universitas Gadjah Mada Press, Yogyakarta. [Indonesian]

Agwu OP, Bakayoko A, Jimoh SO, Jatto SOS, Porembski S. 2020. Influences of land use type and dendrometric variables on fruit production of Garcinia Kola Heckel in Nigeria. Trees For People 2: 1-5. DOI: 10.1016/j.tfp.2020.100015.

Akhmadi NR, Sumarmiyati. 2015. Eksplorasi dan karakterisasi buah kapul (*Baccaurea macrocarpa*) di Kabupaten Kutai Barat, Kalimantan Timur. Pros Sem Nas Masy Biodiv Indon 1: 923-929. DOI: DOI: 10.13057/psnmbi/m010448. [Indonesian]

Aliyah I, Aulia SAS. 2019. The flexibility of parking space of traditional market in the city center (Case Study: Pasar Gede of Surakarta, Indonesia). IOP Conf Ser: Earth Environ Sci 328: 012020. DOI: 10.1088/1755-1315/328/1/012020.

Aliyah I, Setioko B, Pradoto W. 2017. Spatial flexibility in cultural mapping of traditional market area in Surakarta (A case study of Pasar Gede in Surakarta). City Cult Soc 10: 41-51. DOI: 10.1016/j.ccs.2017.05.004.

Angio MH, Irawanto R. 2019. Pendataan jenis buah lokal Indonesia Koleksi Kebun Raya Purwodadi. Jambura Educ Biosfer J 1: 41-46. DOI: 10.34312/jebj.v1i2.2476. [Indonesian]

Apriliani Y, Alam AS, Sulistiawan RSN. 2021. Pengaruh kualitas produk dan harga terhadap keputusan pembelian tomat di Pasar Muka Cianjur. Jurnal Administrasi Bisnis 1: 72-83. DOI: 10.35194/jubis.v1i2.1935. [Indonesian]

Astuthi MMM, Antasari YD. 2020. Persepsi masyarakat terhadap buah jeruk lokal Desa Belantih Kecamatan Kintamani, Kabupaten Bangli. Dwijen AGRO 10: 85-90. DOI: 10.46650/dwijenagro.10.2.1027.85-90. [Indonesian]

Asyfiradayati R, Ningtyas A, Lizansari M, Purwati Y, Winarsih. 2018. Identifikasi kandungan formalin pada bahan pangan (mie basah, bandeng segar dan presto, ikan asin, tahu) di Pasar Gede Kota Surakarta. Jurnal Kesehatan 11: 12-18. DOI: 10.23917/jk.v11i2.7666. [Indonesian]

BPS Indonesia. 2021. Rata-rata Konsumsi Perkapita Seminggu Menurut Kelompok Buah-Buahan Per Kabupaten/kota (Satuan Komoditas). 2021. https://www.bps.go.id/indicator/5/2102/1/rata-rata-konsumsi-perkapita-seminggu-menurut-kelompok-buah-buahan-per-kabupaten-kota. 21 Desember 2022. [Indonesian]

Brooks TM, Pimm SL, Akçakaya HR, Buchanan GM, Butchart SHM, Foden W, Taylor CH, Hoffmann M, Jenkins CN, Joppa L, Li BV, Menon V, Peñuela NO, Rondinini C. 2019. Measuring terrestrial Area of Habitat (AOH) and its utility for the IUCN Red List. Trends Ecol Evol 34: 977-986. DOI: 10.1016/j.tree.2019.06.009.

Davies J, Jordan B, Corrie H, Andrew Z, Nupur J, Patrese A, Allan C, Tom E. 2022. Governance of traditional markets and rural-urban food systems in Sub-Saharan Africa. Habitat Intl 127: 1-10. DOI: 10.1016/j.habitatint.2022.102620.

Deanova AK, Pristiawati CM, Aprilia D, Solikah I, Nurcahyati M, Liza N, Himawan W, Partasismita R, Setyawan AD. 2021. The diversity of edible plants traded in Ir. Soekarno Market, a traditional market in Sukoharjo District, Indonesia. Biodiversitas 22: 4095-4105. DOI: 10.13057/biodiv/d220958.

Dhok A, Butola LK, Anjankar A, Shinde ADR, Kute PK, Jha RK. 2020. Role of vitamins and minerals in improving immunity during Covid-19 pandemic - A review. J Evol Med Dent Sci 9: 2296-2300. DOI: 10.14260/jemds/2020/497.

Dobson MC, Edmondson JL, Warren PH. 2020. Urban food cultivation in the United Kingdom: Quantifying loss of allotment land and identifying potential for restoration. Landsc Urban Plan 199: 1-10. DOI: 10.1016/j.landurbplan.2020.103803.

Ekomadyo AS. 2019. Evaluating revitalized public markets as economic and socio-cultural places in Indonesia. Pertanika J Soc Sci Humanit 27: 963-976.

Esfahlan AJ, Ostadrahimi A, Tabibiazar M, Amarowicz M. 2019. A comparative review on the extraction, antioxidant content and antioxidant potential of different parts of walnut (*Juglans regia* L.) fruit and tree. Molecules 24: 1-40. DOI: 10.3390/molecules24112133.

Faci JM, Blanco O, Medina ET, Martínez-Cob A. 2014. Effect of post veraison regulated deficit irrigation in production and berry quality of autumn royal and crimson table grape cultivars. Agric Water Manag 134: 73-83. DOI: 10.1016/j.agwat.2013.11.009.

Fajar DE, Taryono. 2022. Analisis Pola Perubahan Penggunaan Lahan Pertanian Menjadi Lahan Non Pertanian Menggunakan Metode K-Nearest Neighbour Kabupaten Sukoharjo Tahun 2005 dan 2015. [Skripsi]. Universitas Muhammadiyah Surakarta, Surakarta. [Indonesian]

Fitriani RD, Roviq M, Wardiyati T. 2014. Karakterisasi bunga dan buah mangga hasil persilangan Arumanis-143 (A) X Swarnarika (S), Arumanis-143 (A) X Haden (H) Dan Arumanis-143 (A) X Carabao (C). Buana Sains 14: 95-104. [Indonesian]

Franco FM, Chaw LL, Bakar N, Abas SNH. 2020. Socialising over fruits and vegetables: The biocultural importance of an open-air market in Bandar Seri Begawan, Brunei Darussalam. J Ethnobiol Ethnomed 16: 1-19. DOI: 10.1186/s13002-020-0356-6.

Fuentes S, Hernández-Montes E, Escalona, JM, Bota J, Viejo CG, Poblete-Echeverría C, Tongsona E, Medrano H. 2018. Automated grapevine culticv. classification based on machine learning using leaf morpho-colorimetry, fractal dimension and near-infrared spectroscopy parameters. Comput Electron Agric 151: 311-318. DOI: 10.1016/j.compag.2018.06.035.

Garner BA, Hoban S, Luikart G. 2020. IUCN Red List and the value of integrating genetics. Conserv Genet 21: 795-801. DOI: 10.1007/s10592-020-01301-6.

Gordi I, Mamangkey J, Silalahi M. 2022. The diversity of vegetable and fruit fresh traded in the traditional and modern markets in the Bekasi, West Java and its potential. GSC Biol Pharm Sci 20: 100-109. DOI: 10.30574/gscbps.2022.20.1.0284.

Harris J, Maartenvan Z, Enoch GAD, Babar B, Inge DB, Dhrupad C, Ilsede J, Bartde SP, M. Ehsan D, Luigi G, Roeland K, Sean M, Stepha MM, Marcela Q, Pepijn S. 2022. Fruit and vegetable biodiversity for nutritionally diverse diets: Challenges, opportunities, and knowledge gaps. Glob Food Secur 33: 1-10. DOI: 10.1016/j.gfs.2022.100618.

Harsasto P. 2018. Strategi pembangunan kota berbasis budaya: Revitalisasi Pasar Gede di Kota Surakarta. Jurnal Ilmu Politik 9: 35-46. DOI: 10.14710/politika.9.1.2018.34-46. [Indonesian]

Herlambang RW, Suharto M, Jauhari, Prameswari NS. 2017. Pengenalan Cagar Budaya Pasar Gede Harjonagoro Surakarta bagi generasi muda melalui video time lapse. Jurnal Ilmiah Seni Budaya 2 (2): 130-141. [Indonesian]

Hesthiati E, Priatmodjo D, Wisnubudi G, Sukartono IGS. 2019. Keanekaragaman Hayati Tanaman Buah Langka Indonesia. Lembaga Penerbit Universitas Hasanuddin, Makasar. [Indonesian]

Horak J, Alena P, Andrea P, Lenka S, Petr B, Dusan R, Petr Z. 2013. Biodiversity responses to land use in traditional fruit orchards of a rural agricultural landscape. Agric Ecosyst Environ 178: 71-77. DOI: 10.1016/j.agee.2013.06.020.

Iskandar BS, Iskandar J, Irawan B, Partasasmita R. 2018. Traditional markets and diversity of edible plant trading: Case study in Ujung Berung, Bandung, West Java, Indonesia. Biodiversitas 19: 437-452. DOI: 10.13057/biodiv/d190211.

Iskandar BS, Iskandar J, Mulyanto D, Alfian RL, Suroso S. 2021. Traditional market, social relations, and diversity of edible plants traded in Beringharjo Market, Yogyakarta, Indonesia. Biodiversitas 22: 2045-2057. DOI: 10.13057/biodiv/d220453.

Iskandar BS, Iskandar J, Partasasmita R, Irawan B. 2020. Various medicinal plants traded in the village market of Karangwangi Village, Southern Cianjur, West Java, Indonesia. Biodiversitas 21: 4440-4456. DOI: 10.13057/biodiv/d210963.

Jun Y, Wang Y, Wang Q, Zhang S, Wang M, Feng H, Jiang J, Qiu X, Zhang Y, Chen T. 2022. Generation of fruit and vegetable wastes in the farmers' market and its influencing factors: A case study from Hangzhou, China. Waste Manag 154: 331-339. DOI: 10.1016/j.wasman.2022.10.023.

Kaky E, Gilbert F. 2019. Assessment of the extinction risks of medicinal plants in Egypt under climate change by integrating species distribution models and IUCN Red List criteria. J Arid Environ 170: 1-9. DOI: 10.1016/j.jaridenv.2019.05.016.

Kazemi F, Abolhassani L, Rahmati EA, Amin PS. 2018. Strategic planning for cultivation of fruit trees and shrubs in urban landscapes using the SWOT method: A case study for the city of Mashhad, Iran. Land Use Policy 70: 1-9. DOI: 10.1016/j.landusepol.2017.10.006.

Kementerian Pertanian Republik Indonesia (Indonesian Ministry of Agriculture). 2021. Tingkat Konsumsi Buah Lokal, Sejahterakan Petani. https://www.pertanian.go.id/home/?show=news&act=view&id=4887#:~:text=Rata%2Drata%20konsumsi%20masyarakat%20Indonesia,150%20gram%2Fkapita%2Fhari. 18 Januari 2023. [Indonesian]

Komarayanti S. 2017. Ensiklopedia Buah-buahan lokal berbasis potensi alam Jember. Jurnal Biologi dan Pembelajaran Biologi 2: 61-75. [Indonesian]

Kondo T, Koga K, Sato D. 2020. Effects of nitrogen concentration in fertilizer solution on vegetative growth, flowering, and fruit quality in passion fruit. Trop Agric Dev 64: 161-164. DOI: 10.11248/jsta.64.161.

Kumoro AC, Alhanif M, Wardhani DH. 2020. A critical review on tropical fruits seeds as prospective sources of nutritional and bioactive compounds for functional foods development: A case of Indonesian exotic fruits. Intl J Food Sci 2020: 4051475. DOI: 10.1155/2020/4051475.

Leisner CP. 2020. Review: Climate change impacts on food security-focus on perennial cropping systems and nutritional value. Plant Sci 293: 1-7. DOI: 10.1016/j.plantsci.2020.110412.

Lestari 2014. Morphological variation and species distribution of *Baccaurea dulcis* (Jack) Müll. Arg. in West Java, Indonesia. Intl J Biol 6: 17-28. DOI: 10.5539/ijb.v6n1p17.

Noverian W, Elfrida, Suwardi AB, Mubarak A.2020. Inventarisasi jenis buah-buahan lokal sebagai sumber pangan bagi masyarakat Lokop Aceh Timur. Jurnal Jeumpa 7: 319-327. DOI: 10.33059/jj.v7i1.2956. [Indonesian]

Nugroho AAS, Bowo C, Sudibya J. 2019. Indeks kekeringan SPI (Standardized Precipitation Index) dan pengaruhnya terhadap produktivitas hortikultura tahunan di Kabupaten Jember. Berkala Ilmiah Pertanian 2: 149-154. DOI: 10.19184/bip.v2i4.16312. [Indonesian]

Nurshillah C, Anggorowati D, Putri ER, Balgis M, Nurwulandari M, Murtiningsih, Agustina N, Wulandari P, Liza N, Himawan W,

Setyawan AD. 2022. Diversity of edible plants traded in Legi Traditional Market, Surakarta, Indonesia. Asian J Ethnobiol 5: 52-61. DOI: 10.13057/asianjethnobiol/y050106.

Panagos P, Standardi G, Borrelli P, Lugato E, Montanarella L, Bosello F. 2018. Cost of agricultural productivity loss due to soil erosion in the European Union: From direct cost evaluation approaches to the use of macroeconomic models. Land Degrad Dev 29: 471-484. DOI: 10.1002/ldr.2879.

Pedreschi R, Hollak S, Harkema H, Otma E, Robledo P, Westra E, Somhorst D, Ferreyra R, Defilippi BG. 2016. Impact of postharvest ripening strategies on 'Hass' avocado fatty acid profiles. S Afr J Bot 103: 32-35. DOI: 10.1016/j.sajb.2015.09.012.

Pratama MF, Dwiartama A, Rosleine D, Abdulharis R, Irsyam ASD. 2019. Documentation of Underutilized Fruit Trees (UFTs) across indigenous communities in West Java, Indonesia. Biodiversitas 20: 2603-2611. DOI: 10.13057/biodiv/d200924.

Priyambodo CS, Sastryawanto H, Hermawati DT. 2019. Analisis preferensi konsumen buah jeruk di Pasar Keputran Utara, Surabaya. Jurnal Ilmiah Sosio 19: 85-103. [Indonesian]

Puteri LCP, Fajarwati A. 2016. Pengaruh eksistensi pasar tradisional terhadap perkembangan wilayah Kota Surakarta. Jurnal Bumi Indonesia 5: 1-9. [Indonesian]

Putra AK, Sukmono A, Sasmito B. 2018. Analisis hubungan perubahan tutupan lahan terhadap suhu permukaan terkait fenomena urban heat island menggunakan citra landsat (Studi Kasus: Kota Surakarta). Jurnal Geodesi Undip 7 (3): 22-31. [Indonesian]

Rada P, Josef PH, Jaroslav H, Karolína M, Jakub H. 2022. Urban fruit orchards: Biodiversity and management restoration effects in the context of land use. Urban For Urban Green 75: 1-10. DOI: 10.1016/j.ufug.2022.127686.

Rahayu JN, Fauziyah E, Ariyani AHM. 2012. Preferensi konsumen terhadap buah apel impor di Toko Buah Hokky dan Pasar Tradisional Ampel Surabaya. Agriekonomika 1: 52-67. DOI: 10.21107/agriekonomika/v11350. [Indonesian]

Rudarmono R, Ardiarini NR, Waluyo B, Ashari S. 2022. Interspecific and intraspecific cross-compatibility of *Durio kutejensis* and hybrid *Durio zibethinus x kutejensis*. Biodiversitas 23: 1837-1843. DOI: 10.13057/biodiv/d230416.

Ruwaida IP, Santosa E, Susila AD, Widodo WD, Wachjar A, Nasrullah N. 2022. Perceived biodiversity of fruit species for urban greenery in Indonesia: Case studies in Bogor, Jakarta, and Yogyakarta. Biodiversitas 23: 3166-3177. DOI: 10.13057/biodiv/d230644.

Safitri RA, Nurdiani S, Riana D, Hadianti S. 2019. Klasifikasi jenis buah apel menggunakan metode Orde 1 dengan Algoritma Multi Support-Vector Machines. Paradigma - Jurnal Informatika dan Komputer 21: 167-172. DOI: 10.31294/p.v21i2.6526. [Indonesian]

Saputra DF. 2021. Evaluasi Kesesuaian Penggunaan Lahan Kota Surakarta Tahun 2011-2020 Terhadap Rencana Tata Ruang Wilayah Kota Surakarta Tahun 2011-2030. [Skripsi]. Universitas Muhammadiyah Surakarta, Surakarta. [Indonesian]

Sarvina Y, Sari K. 2017. Dampak ENSO terhadap produksi dan puncak panen durian di Indonesia. Jurnal Tanah dan Iklim 41: 147-155. [Indonesian]

Setiawan AA, Utami DP, Hasanah U. 2019. Keputusan konsumen dalam mengkonsumsi buah lokal dan buah impor di Kabupaten Purworejo. Surya Agritama 8: 165-172. [Indonesian]

Shivran JS, Jat ML, Jat RK, Jat A. 2020. Adoption of regular bearing in mango over biennial bearing. Intl J Curr Microbiol Appl Sci 9: 149-154. DOI: 10.20546/ijcmas.2020.905.016.

Simchon EB, Grunwald Y, Ari GB, Rosenfeld A, Shelef O. 2022. A village a field? Agronomic evaluation of fruit trees in inhabited space - lessons for land use policy from a case study in Israel's Sharon Region. Land Use Policy 123: 1-10. DOI: 10.1016/j.landusepol.2022.106411.

Soebiyan V, Saragih JB, Wondoamiseno K. 2020. Model development of Pasar Gedhe Hardjonegoro, Surakarta for sustainable tourism. IOP Conf Ser: Earth Environ Sci 426: 012086 DOI: 10.1088/1755-1315/426/1/012086.

Sujarwo W, Lugrayasa IN, Kuswantoro F. 2018. Studi etnobotani tiga pasar tradisional di Kabupaten Tabanan Bali. Jurnal Ilmu-Ilmu Hayati 17: 283-297. DOI: 10.14203/beritabiologi.v17i3.3342. [Indonesian]

Sulaini AA, Sabran SF. 2018. Edible and medicinal plants sold at selected local markets in Batu Pahat, Johor, Malaysia. AIP Conf Proceed 2002 (1): 020006. DOI:10.1063/1.5050102.

Suwardi AB, Navia ZI, Harmawan T, Syamsuardi, Mukhtar E. 2020. Ethnobotany and conservation of indigenous edible fruit plants in South Aceh, Indonesia. Biodiversitas 21: 1850-1860. DOI: 10.13057/biodiv/d210511.

Tamang B, Sarkar BC, Pala NA, Shukla G, Vineeta, Patra PS, Bhat JA, Dey AN, Chakravarty S. 2019. Uses and ecosystem services of Trees Outside Forest (TOF)-A case study from Uttar Banga Krishi Viswavidyalaya, West Bengal, India. Acta Ecologica Sinica 39: 431-437. DOI: 10.1016/j.chnaes.2018.09.017.

Vlkova M, Verner V, Kandakov A, Polesny Z, Karabaev N, Pawera L, Nadvornikova I, Banout J. 2015. Edible plants sold on marginal rural markets in Fergana Valley, southern Kyrgyzstan. Bulg J Agricult Sci 21: 243-50.

Widiatama A, Karyanto A, Rugayah R, Widagdo S. 2021. Pengaruh pemberian Paclobutrazol dan pupuk terhadap induksi pembungaan manggis (*Garcinia mangostana* L.). Jurnal Agrotek Tropika 9: 313-320. DOI: 10.23960/jat.v9i2.5024. [Indonesian]

Yuliawati NWP, Wiraatmaja IW, Yuswanti H. 2016. Identifikasi dan karakterisasi sumber daya genetik tanaman buah-buahan lokal di Kabupaten Gianyar. E-Jurnal Agroekoteknologi Tropika 5: 297-309. [Indonesian]