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Morpho-physiological-based selection criteria for chili (*Capsicum annuum*) under drought stress during vegetative to generative phase

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Abstract. *Lestari P, Syukur M, Trikoesoemaningtyas, Widiyono W. 2023. Morpho-physiological-based selection criteria for chili (*Capsicum annuum*) under drought stress during vegetative to generative phase. Biodiversitas 24: 2315-2323.* Chili (*Capsicum annuum* L.) is a drought-sensitive plant. Improving chili yield under droughts requires understanding of genetic variability (GCV), heritability (h^2bs), and traits associated with yield. This study aims to determine the variability and heritability of plant traits and explore their relationships and path toward yield under drought stress during the vegetative to generative phases. The six Indonesian chili genotypes, namely Genie, Adelina, C5, SSP, Viola, and Anies, were studied. The experiment was laid out following a completely randomized block design. The media moisture content acted as the main plot (35% field capacity (FC), 50% FC, 80% FC), and six chili genotypes as the subplot. Variance analysis revealed that the broadest GCV was recorded in 35% FC for yield (94.04), fruit numbers (FN) (117.20), and the other six characters. Considering h^2bs, correlation, and path coefficient analyses, FN (h^2bs=95.43; r=-0.82; C=-0.60) and fruit length (h^2bs=99.64; r=0.60; C=0.22) effective for increasing the yield at 80% FC, while FN (h^2bs=98.32; r=0.54; C=0.65) and leaf area (h^2bs=97.09; r=0.85; C=0.48) were effective at 35% FC. This result implies the importance of these characteristics as the selection criteria for crop improvement under drought stress.

Keywords: *Capsicum annuum*, correlation, heritability, path coefficient, selection criteria

Abbreviation: GCV: Genotypic coefficient variation; PCV: phenotypic coefficient variation; h^2bs: broad-sense heritability

INTRODUCTION

The continual droughts and increasing water shortages in agricultural environments have a significant negative impact on yields for numerous crops, including chili. Drought disrupts gas exchange in plant tissues by affecting the role of stomata (Campos et al. 2014; Widuri et al. 2020) and trichome density (González-Klenner et al. 2022). It also induces oxidative stress, leading to growth retardation, delayed flowering, flower abortion, and reduced fruit set. These effects ultimately decrease plant biomass and yield (Malika et al. 2019; Widuri et al. 2020).

Capsicum annuum L. is a chili species widely cultivated in Indonesia and the globe for its fresh and processed products (Lestari 2021). Chili is a sensitive plant when it comes to drought. Drought stress during the vegetative stage interferes with organ development and plant growth (Widiyono dan Hidayati 2005; Widiyono 2016), resulting in a 30-50% reduction in yield (Ichwan et al. 2017). However, severe drought stress in the flowering phase can lead to chili yield losses ranging from 45.9% to 100% (Rosmaina et al. 2019a; Saendamuen et al. 2022). Unfortunately, studies have been restricted to each growth phase, and simultaneous observations from vegetative to generative phases are needed to complete our understanding of drought stress's effects at each chili growth phase.

Plant resistance under drought conditions has become the most important concern of farmers and breeders to increase yields on limited irrigation for the last decade (Luo et al. 2019). Using tolerant chilies under drought conditions in the tropics can significantly decrease production costs and improve plant productivity (Chaturvedi and Srinivas 2019). While breeding based on molecular markers is developed, gene selection based on phenotypic characterization is still relied upon plant breeding (Passioura 2012; Luo et al. 2019). Selection criteria are required in breeding programs to identify genotypes with high yields under stressful conditions. Appropriate selection criteria will provide the screening process for tolerant genotypes under stress conditions effective and efficient.

Understanding genetic variability and determining yield-related tolerance characteristics are essential for finding the appropriate selection criteria and increasing the probability of obtaining high-yielding and drought-tolerant chili genotypes under drought-stress conditions. Correlation and pathway coefficient are proven successful statistical analyses in identifying key characteristics in many species, including soybean (Kuswantoro 2017), tomato (Saleem et al. 2013; Ritonga et al. 2018), and chili

(Malika et al. 2019; Mahmood et al. 2021). Fruit length and diameter, capsaicin contents, and fruit quantity have been identified as selection criteria for chilies under drought in the generative phase (Rosmaina et al. 2019b; Mahmood et al. 2021). Meanwhile, the shoot-root ratio and leaf area has been noted as helpful selection criteria for drought in the vegetative phase (Widuri et al. 2020). However, information about genetic variability, heritability, and criteria selection for drought tolerance traits during the vegetative to generative phase has not been reported in chili research. This study aims to determine the genetic variability and heritability of plant traits, explore their relationships, and analyze the path coefficient to yield under drought stress during the vegetative to generative phases. These characteristics will be used as selection criteria for chili crop improvement under drought stress during the vegetative to generative phase.

MATERIALS AND METHODS

Study area and genetic materials

The research was conducted at the Research Center for Biology-Indonesian Institute of Sciences (LIPI), now the National Research and Innovation Agency (BRIN), Cibinong, Indonesia, February to August 2021. The drought simulation started in March, at the beginning of the dry season in that year. The experiment site was located at 6°29'52.9"S 106°50'43.4"E, 250 m above sea level (GPS Garmin 64s, Germany). The daily temperature and air humidity ranged from 32° to 39°C and 38 to 68%, respectively (AS ONE 2-897-01 BT-3, Japan), and the light intensity in the screen house was between 12,000 and 27,000 lux during the day (LX 1010 B Digital Lux Meter, Japan). Genetic materials in this experiment consisted of six Indonesian *C. annuum* genotypes: Genie, Adelina, C5, SSP, Viola, and Anies (Table 1).

Procedures

Seeds from each chili genotype were surface-sterilized for 10 min with commercial fungicide (0.5% concentration of active mancozeb 80%) and then rinsed for 30 min under tap water. The seeds were then sown in a seedling tray containing a mix of rice husk-charcoal, compost, and manure (1:1:1 v/v/v ratio). Healthy and uniform 28-day-old seedlings (about 4-6 leaves) were transplanted into polybags 40x40 cm (equal to 50.240 cm^3 media) and subjected to drought until each genotype flowered in each treatment. Similar growing media was used as a growing substrate. Each chili genotype was laid following the randomly complete block design. The replication is nested in water regimes. Each replication consisted of three plants, with one plant per polybag. Drought was imposed by maintaining the media water content at 50% Field Capacity (50% FC) (Equal to 1.46 MPa) as moderate drought stress and 35% FC (2.25 MPa) as severe drought stress, respectively (Okunlola et al. 2017; Lestari et al. 2023). The 80% FC (0.47 MPa) was used as a control to prevent the chili plants' roots from being submerged (Mardaninejad et al. 2017). Drought treatment was applied from transplanting to the flowering phase to avoid differences in response between genotypes due to growth phase variations. Then, the treated plants were watered daily to let them recover until the latest-flowering genotype had been harvested four times to predict each treatment's actual fruit production. Daily air temperature, air relative humidity, and light intensity were recorded manually.

Field Capacity (FC) refers to the moisture content of soil after free water has been lost through gravity. The macropores of the media contain oxygen, while the micropores contain water (Lopez and Barclay 2017). The water content at field capacity was determined referred to Lestari et al. (2023). The planting media was saturated with a particular volume of water. It then left for 48 hours until no water dripped from the media. The media was weighed as weight at field capacity (7200 g/polybag). The planting medium was then oven-dried at 60°C to a constant weight and re-weighed (3900 g/polybag). The moisture content at field capacity was calculated as the difference in the weight of the media at field capacity and oven-dried media (7200 g/polybag-3200 g/polybag=4000 g/polybag). The weight of planting medium at 80% FC and 35% FC is calculated based on the volume of water at field capacity using the formula described by Lestari et al. (2023) as follows: (i) Weight of medium at FC 80% (g/polybag) = (80% × water content at field capacity) + weight of oven-dried media. (ii) Weight of medium at FC 50% (g/polybag) = (50% × water content at field capacity) + weight of oven-dried media. (iii) Weight of medium at FC 35% (g/polybag) = (35% × water content at field capacity) + weight of oven-dried media.

To ensure uniform initial media moisture content, each polybag was saturated a month before the drought treatment and left to dry naturally until the media water content was below 35% FC. During transplanting, a specific volume of water was added according to the drought treatment. The polybag weight of each treatment medium was maintained by adding water every two days. The water loss rate was determined daily using nine polybags without plants (Lu et al. 2018).

Morpho-physiological measurements

The plant response to drought was evaluated by comparing 22 variables, including morphological and physiological characteristics, under untreated, moderate, and severe drought stress during the drought treatment. The treated plants were well-watered daily after exposure to drought until the end of the experiment. Plant height (PHV and PHG) (cm), leaf area (LAV and LAG) (cm^2), and chlorophyll content (CHLV and CHLG) (SPAD) were measured at vegetative stage (28-40 days after sowing, DAS) and generative stage (45-65 DAS) for each genotype (Okunlola et al. 2016). The total chlorophyll content was estimated using the Konika Minolta Chlorophyll Meter SPAD-502 Plus (Tokyo, Japan) (Jiang et al. 2017).

Table 1. Morphological characters of six chili genotypes and their drought tolerance information

Genotype	Description	Drought Tolerance	Source	Reference
Genie	Bird chili, plant height: 75-85 cm, fruit length: 4-4.5 cm[1]	Tolerant[2,5]	Benih Citra Asia	[1]Ministry of Agriculture (2018),
Adelina	Red chili, ornamental chili, plant height: 75-90, dichotomy height: 17.33 cm, immature fruit color: purple, fruit length: 8.03 cm[4]	-	IPB	[2]Widuri et al. (2020),
C5	Red chili, plant height: 75.77 cm, dichotomy height: 25.33 cm, fruit length: 10.67 cm[4]	Tolerant[3]	IPB	[3]Millah et al. (2021),
SSP	Curly chili, plant height: 66.80 cm, fruit length: 12.72 cm[1]	Tolerant[3]	IPB	[4]Sahid et al. (2022);
Viola	Bird chili, ornamental chili, plant height: 63.67 cm, dichotomy height: 12 cm, early-flowering, immature fruit color: violet, fruit length: 4.5-5 cm[4]	-	IPB	[5]Lestari et al. (2023)
Anies IPB	Red chili, plant height: 44.20-68.27 cm, dichotomy height: 17.23-21.16 cm, fruit length: 12.02-19.35 cm[1]	Sensitive[3]	IPB	

Plant biomass was estimated by measuring the dry weight of the root (RB), leaves (LB), and stem (SB) at the end of drought treatments. The above-ground part (leaves and stem) and root were oven-dried at 50°C to a constant weight and then weighed to obtain shoot and root dry-weight (g). The root weight ratio to total biomass (RPAR) (%) was calculated by dividing the root dry weight by the total plant dry weight, while the shoot-root ratio (SR) was determined by dividing the plant's above-ground dry weight (leaf and stem) by root dry weight (Comas et al. 2013). Flowering time (FT) was determined when 50% of each genotype blossomed (Week after transplanting, WAT). The total fruit weight (TFW) in grams and the total number of fruits per plant (FN) were accumulated from all harvested fruit during the experiment periods. Water Use Efficiency for fruit fresh weight (WUEf) was calculated based on total fruit fresh weight divided by total water irrigated during the experiment (Widiyono dan Nugroho 2023). Fruit length (FL) and fruit diameter (FD) in cm were measured from five fruit samples at the second to the third harvest of each treatment. The leaf number (LN) and leaf water potential (WP) in MPa were observed at the generative phase. Leaf and media water potential was measured using WP4 Dewpoint Potentiometer (Widiyono et al. 2020).

Epidermal imprint microscopy and measurements

An unfolded fourth leaf from the apex of three plants from each treatment was collected for epidermal imprint microscopy using the following step (Koch et al. 2019): The abaxial surface of the leaf was covered with a thin coat of clear nail polish (Revlon Double Twist, Revlon, New York, USA), avoiding the leaf midrib. For each leaf, the epidermal cell area was obtained by drawing cells at the base, middle, and at the tip of a leaflet. The imprinted layers were delicately transferred to glass slides and laminated using transparent plastic tape. A digital camera (Indomicroview ver. 3.7, Indomicro, Jakarta, Indonesia) coupled with an Olympus CX31 microscope (Olympus, Tokyo, Japan) was used to photograph the slides at 400x magnification. From each slide, three representative images were selected to observe the stomata length (SL) and cell area (CA). Leaf area (LA) was calculated using ImageJ (https://imagej.nih.gov/ij/) from a scan of the leaf blade recorded using a flatbed scanner HP Scanjet G3010 (Hewlett-Packard, California, USA) (Lestari et al. 2023). The imageJ application was also used to estimate stomatal length (SL) in µm; choose and trace an outline around the perimeter of at least 50 pavements, including guard cells, to estimate the size of each cell. The total area of the chosen cells was divided by the quantity of pavement and guard cells to determine the average cell area in μm^2.

Data analysis

All data were compiled and tabulated using Microsoft Excel Office 2019. The evaluation of plant characteristics under each water regime was performed using analysis of variance followed by the Tukey Honestly Significant Difference (HSD) post hoc test at pVal <0.05, using an online tool (http://pbstat.com/pkbt-stat/). Genotypic (Vg) and phenotypic (Vp) variability were estimated by extracting the variance component in ANOVA (Syukur et al. 2010). Genotypic coefficient of variation (GCV) and Phenotypic coefficient of variation (PCV) were evaluated according to Lush (1949) as follows:

(i) GCV = (Vg/X) x 100%; PCV = (Vp/X) x 100%
(ii) Vg = (MSg-MSe)/r; Vp = Vg + (MSe/r),
(iii) h^2bs = Vg/Vp x 100%

MSg and MSe are the mean squares for genotype and error, respectively. Vg is the genotypic variance, and Vp is the phenotypic variance. r is the number of replications. X is the general mean of all genotypes. The GCV and PCV were classified as low (<10%), moderate (10-20%), and high (>20%) following Lush (1949). Broad-sense heritability (h^2bs) was estimated according to Johnson and Robinson (1955). Heritability values were categorized as low (<20%), moderate (20-50%), and high (>50%) following Syukur et al. (Syukur *et al.* 2010). The phenotypic correlation coefficient was calculated based on Pearson correlation by standard procedures (Johnson dan Robinson 1955). The significant-correlated characters to yield were further partitioned into direct and indirect effects by path analysis using Minitab 18 software (www.minitab.com), according to IRRI (International Rice Research Institute 2006).

RESULTS AND DISCUSSION

Drought effect on morpho-physiological characteristics and yield component

The applied drought stress in this study successfully caused variations in leaf water potential (WP) since the vegetative phase, indicated by the diminishing leaf water potential with increasing severity of drought stress (Figure 1C). Our study demonstrated that moderate drought stress (50% Field Capacity, FC) reduced plant height (PHG) and leaf area (LAG) particularly on the generative phase, while severe drought stress (35% FC) had effect on both variables (PHV and LAV) since the vegetative phase (Figures 1A, 1B, and 1D), that similarly on curly chili presented by Widuri et al. (2020). The study also found that other leaf parameters, such as cell area (CA) and stomatal length (SL) were detected to decrease in the generative phase (Figure 1D), that accordance with research in bird-eye chili by (Lestari *et al.* 2023). However, drought had less effect on chlorophyll contents throughout both the vegetative (CHLV) and generative phases (CHLG) (Figures 1A and 1D). At the end of the stress treatment, the dry weight of above-ground organs decreased more than the below-ground organs (roots) (Figures 1B and 1C), that is suitable on chilies research as presented by Widuri et al. (2020) and Lestari et al. (2023).

The research resulted that drought negatively affect to above-ground organs, explained by leaves (LB) and stem dry weight (SB). The decreasing of above-ground organs in severe drought conditions effect to reduce the shoot-root ratio (SR) and increase RPAR in severe drought conditions (Figure 1C).

Drought caused the abortion of early flower buds, as previously reported on chilies by Malika et al. (2019) and Rosmaina et al. (2019b), delay flowering time (Figure 1E), led to a decrease in fruit size and number and resulted in decreasing of the total fruit weight (Figure 1E). The yield-reduction of six genotypes reached 37% when subjected to moderate drought stress and 63% for severe drought stress. This study also explained that the six chili genotypes used in the study varied in plant height, leaf size, and fruit shape (Table 1). The broad variations in plant canopy and fruit characteristics among the chili genotypes studied were illustrated by the high standard deviation for RPAR, root length (RL), leaf number (LN), plant height at the generative phase (PHG), and yield (TFW) (Figure 1). Genetic variability was detected in this study through PCV and GCV estimations.

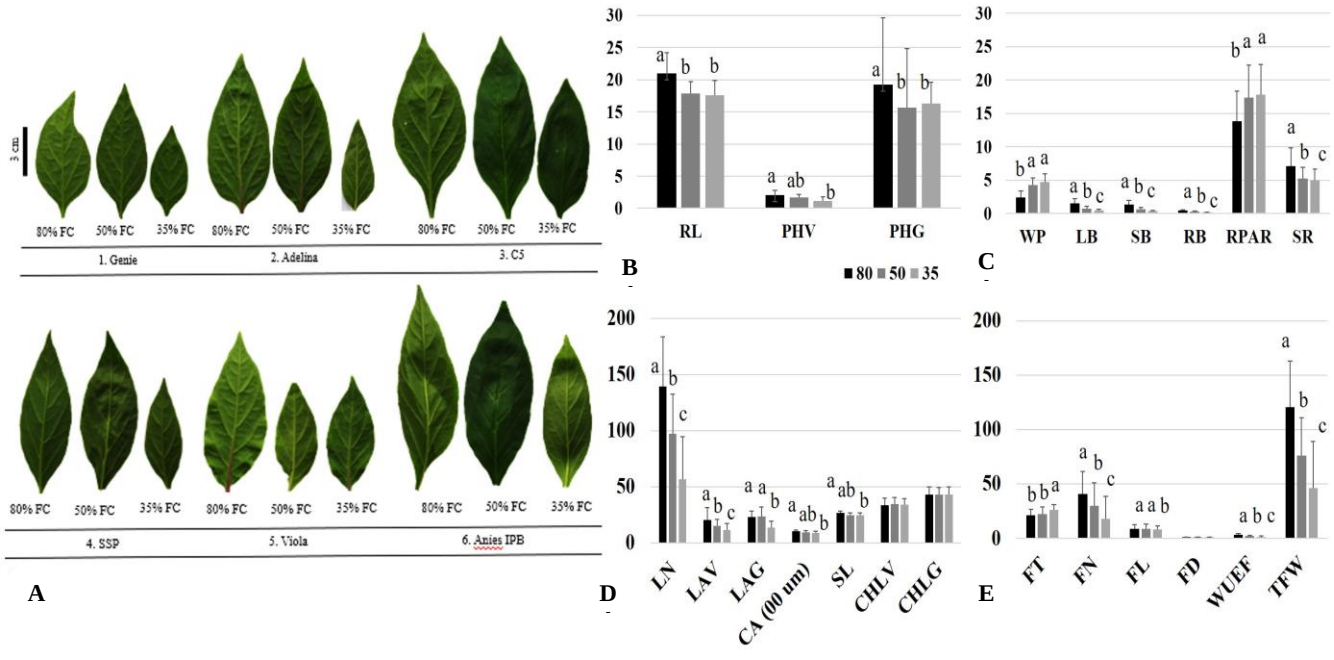

Figure 1. Stratified drought effect on chili. A. Leaf representative of six chili genotypes on several water regimes. B, C, D, E. chili plant responded to morpho-physiological traits. RL: root length (cm); PHV: plant height at vegetative phase (cm); PHG: plant height at generative phase (cm); LN: leaves number; LAV: leaf area at vegetative phase (cm^2); LAG: leaf area at generative phase (cm^2); CHLV: leaf chlorophyll content at vegetative phase (SPAD); CHLG: leaf chlorophyll content at generative phase (SPAD); CA: cell area (μm^2); SL: stomatal length (µm); WP: leaf water potential (MPa); LB: leaves dry weight (g); SB: stem dry weight (g); RB: root dry weight (g); RPAR: root biomass proportion to total dry weight (%); SR: shoot-root ratio; FT: day to flowering (WAP); FN: number of fruit; FL: fruit length (cm); FD: fruit diameter (cm); WUEf: water use efficiency based on fruit fresh weight (g L^{-1}); TFW: total fruit weight per plant (g). Bar means STDEV. Bar followed with the same letter in the same group are not different based on the 5% HSD test

Genetic variability under stratified drought stress

In this study, genetic variability is expressed by Means of Square value for genotype (MSg) and error (MSe) (Table 2), phenotypic coefficient of variation (PCV), and genotypic coefficient of variation (GCV) (Table 3). In our investigation, the MSg value was generally higher than the MSe value in almost all characters (Table 2), indicating that the genetic factors have a more significant impact than environmental factors in regulating phenotypic traits. However, for water potential (WP) and root dry weight (RB), the MSe (1.87 and 0) was greater than the MSg (0.95 and 0), indicating that environmental factors play a more significant role in these traits. The genotype value was not statistically significant for either variable at 35% FC, resulting in a broad-sense heritability value was 0.00. (Table 3). In contrast, fruit diameter (FD), water use efficiency per fruit fresh weight (WUEf), and dry weight of leaves and stems (LB and SB) all have a heritability value was 100 under 35% FC, showing that genetic factors control these traits significantly (Table 3).

The GCV and PCV values for most traits were recorded in the broad category based on Lush's classification (1949) for each water regime, indicating a high degree of genetic variation. However, root length (RL), chlorophyll at vegetative and generative (CHLV and CHLG), cell area (CA), and stomatal length (SL) exhibited lower values, suggesting a lower degree of genetic variation. GCV and PCV values were the highest at 35% FC for yield (TFW) (94.04 and 95.61), total number of fruit (FN) (117.20 and 118.20), WUEf (88.88 and 90.40), fruit length (FL) (46.58 and 46.70), and the other six traits in vegetative organs, such as RL, number of leaves (LN), leaf area at generative phase (LAG), CHLG, and SL (Table 3), supported by the highest in the broad-sense heritability category. The highest GCV in 35% FC strongly suggests the existence of broad genetic variability under such drought-related conditions (Saleem et al. 2013; Ritonga et al. 2018). These variables were also greatly influenced by additive and epistatic gene effects (Usman et al. 2014), as seen by the high category in heritability values at 35% FC. Selection in breeding programs for improving those traits would be effective in early generations (F2-F3) (Mawasid et al. 2019). So, it is strongly assumed that 35% FC is the most suitable environment to determine the yield-based tolerance level on *C. annuum* in this investigation. Based on these considerations, correlation analysis and path coefficient were only carried out at 80% FC and 35% FC.

Table 2. Estimate of means square for genotype (MSg) and error (MSe) under different water regimes

Variables	Means Square for genotype (MSg)			Means Square for error (MSe)			P<F value		
	80% FC	50% FC	35% FC	80% FC	50% FC	35% FC	80% FC	50% FC	35% FC
RL	15.1	5.32	12.43	6.09	2.79	2.79	ns	ns	*
PHV	1.77	0.35	0.54	0.15	0.22	0.2	**	ns	ns
PHG	357.93	282.26	26.81	5.56	1.33	3.23	**	**	**
LN	5562.07	3716.19	4724.55	395	183.05	59.47	**	**	**
LAV	409.18	46.12	66.97	5.95	22.18	22.28	**	ns	ns
LAG	48.37	189.49	94.87	8.82	15.64	2.76	*	**	**
CHLV	124.71	95.52	82.69	3.05	1.49	3.25	**	**	**
CHLG	142.94	102.07	147.23	13.71	8.72	4.84	**	**	**
WP	3.13	3.22	0.95	0.24	0.36	1.87	**	**	ns
LB	1.27	0.31	0.08	0.2	0.01	0	**	**	**
SB	1.14	0.18	0.05	0.1	0.01	0	**	**	**
RB	0.04	0.01	0	0.01	0	0	**	**	**
RPAR	65.4	67.31	64.47	2.33	4.78	2.02	**	**	**
SR	22.74	8.39	8.25	1.52	0.36	0.29	**	**	**
CA	33397.83	85891.71	64998.19	17705.17	9873.64	7152.29	ns	**	**
SL	5.07	6.27	8.17	2.13	2.07	2.28	ns	ns	*
FT	76.1	113.22	54.27	6.73	4.98	13.38	**	**	*
FN	1276.18	1367.66	1369.21	58.37	48.61	23.02	**	**	**
FL	47.79	52.84	42.86	0.17	0.5	0.21	**	**	**
FD	0.3	0.48	0.26	0.02	0.03	0	**	**	**
WUEf	3,590.40	1,742.81	3,839.54	355.35	217.84	128.87	**	**	**
TFW	4878.79	3064.06	5848.44	483.5	279.94	190.43	**	**	**

Note: **Significant at 1%; *Significant at 5%; ns: nonsignificant based on ANOVA test. RL: root length (cm); PHV: plant height at vegetative phase (cm); PHG: plant height at generative phase (cm); LN: leaves number; LAV: leaf area at vegetative phase (cm^2); LAG: leaf area at generative phase (cm^2); CHLV: leaf chlorophyll content at vegetative phase (SPAD); CHLG: leaf chlorophyll content at generative phase (SPAD); WP: leaf water potential (MPa); LB: leaves dry weight (g); SB: stem dry weight (g); RB: root dry weight (g), RPAR: root biomass proportion to total dry weight (%); SR: shoot-root ratio; CA: cell area (μm^2); SL: stomatal length (μm); FT: flowering time (WAP); FN: number of fruit; FL: fruit length (cm); FD: fruit diameter (cm); WUEf: water use efficiency based on fruit fresh weight ($g\ L^{-1}$); TFW: total fruit weight per plant (g)

Table 3. Estimates of GCV, PCV, and broad-sense heritability under different water regimes

Variables	GCV			PCV			Broad-sense heritability		
	80%FC	50%FC	35%FC	80%FC	50%FC	35%FC	80%FC	50%FC	35%FC
RL	8.25	5.14	10.22	10.68	7.46	11.60	59.67	47.56	77.55
PHV	36.32	12.32	29.19	37.96	20.21	36.79	91.53	37.14	62.96
PHG	56.48	61.93	17.23	56.92	62.08	18.38	98.45	99.53	87.95
LN	29.73	35.18	69.04	30.85	36.08	69.47	92.90	95.07	98.74
LAV	56.00	18.11	32.45	56.41	25.14	39.72	98.55	51.91	66.73
LAG	15.48	31.88	39.90	17.12	33.29	40.49	81.77	91.75	97.09
CHLV	18.93	15.97	15.05	19.17	16.10	15.35	97.55	98.44	96.07
CHLG	15.24	12.86	16.04	16.02	13.45	16.31	90.41	91.46	96.71
WP	41.48	23.15	0.00	43.17	24.56	11.96	92.33	88.82	0.00
LB	38.60	43.65	36.24	42.05	44.37	36.24	84.25	96.77	100.00
SB	43.96	38.33	39.32	46.02	39.44	39.32	91.23	94.44	100.00
RB	23.84	22.02	0.00	27.53	22.02	0.00	75.00	100.00	0.00
RPAR	33.27	26.33	25.63	33.88	27.32	26.04	96.44	92.90	96.87
SR	37.68	31.37	32.60	39.01	32.06	33.19	93.32	95.71	96.48
CA	6.98	16.69	15.74	10.19	17.74	16.68	46.99	88.50	89.00
SL	3.68	4.76	5.66	4.84	5.82	6.67	57.99	66.99	72.09
FT	22.48	26.58	14.02	23.55	27.18	16.15	91.16	95.60	75.35
FN	49.14	69.41	117.20	50.30	70.68	118.20	95.43	96.45	98.32
FL	45.50	46.66	46.58	45.59	46.88	46.70	99.64	99.05	99.51
FD	27.84	37.52	26.94	28.82	38.75	26.94	93.33	93.75	100.00
WUEf	31.64	34.03	88.88	33.33	36.38	90.40	90.09	87.53	100.00
TFW	31.64	39.94	94.04	33.33	41.90	95.61	90.09	90.86	96.74

Note: GCV: genotypic coefficient variance; PCV: phenotypic coefficient variance. The GCV and PCV were classified as low (<10%), moderate (10-20%), and high (>20%). RL: root length (cm); PHV: plant height at vegetative phase (cm); PHG: plant height at generative phase (cm); LN: leaves number; LAV: leaf area at vegetative phase (cm^2); LAG: leaf area at generative phase (cm^2); CHLV: leaf chlorophyll content at vegetative phase (SPAD); CHLG: leaf chlorophyll content at generative phase (SPAD); WP: leaf water potential (MPa); LB: leaves dry weight (g); SB: stem dry weight (g); RB: root dry weight (g); RPAR: root biomass proportion to total dry weight (%); SR: shoot-root ratio; CA: cell area (μm^2); SL: stomatal length (µm); FT: day to flowering (WAP); FN: number of fruit; FL: fruit length (cm); FD: fruit diameter (cm); WUEf: water use efficiency based on fruit fresh weight (gL^{-1}); TFW: total fruit weight per plant (g)

Correlation and path analysis

The correlation between yield and yield-contributing characteristics is crucial in plant breeding programs since it is a tool to estimate yield improvement through other traits (International Rice Research Institute 2006). Selection criteria through yield and yield-contributing characters help to identify high-yield coupled with drought-tolerant genotypes. The correlation coefficient between two characters ranges from 0 to 1. The relationship between the two characters is highly correlated when the coefficient correlation is closer to 1. Conversely, when the coefficient is close to 0, the relationship is weakly correlated (Singh dan Chaudhary 2007). However, correlation merely reflects the mutual link between two features and cannot describe a causal relationship, so selection based on correlation needs to be supported by pathway analysis (International Rice Research Institute 2006).

Pathway analysis is a kind of multivariate analysis, a type of regression that partitions the relationship between yield and yield-contributing characters into direct and indirect effects in a linear relationship (Singh dan Chaudhary 2007). Combining correlation and path coefficient has been proven to increase the accuracy of predicting selection criteria for tolerance to environmental stress in wheat (Mohammadi 2018), tomato (Ritonga et al. 2018), and chilies (Malika et al. 2019; Rosmaina et al. 2019b). In our research, correlation and path analysis was done only under optimum (80% FC) and severe drought stress conditions (35% FC) as the selected environment.

Under optimum conditions, the yield (TFW) was strongly correlated (Pvalue <0.01) with yield components: FN (-0.82), FL (0.60), and FD (0.62). In addition, yield was also correlated with leaf area at the vegetative phase (LAV) (0.69), root dry weight (RB) (0.57), and water potential (WP) (0.55) in this research (Figure 2A). However, only WP and FN contributed directly to yield at these conditions (Figure 2B) (R square=0.8176; Residu=0.4271), while LAV and RB influenced the yield through FN. Lopez and Barclay (2017) and Zakaria et al. (2020) have well-documented the leaves and root contribution to yield via source-sink interaction in many species. The fruit serves as a sink organ that absorbs photosynthesis products. Large leaves area and deep roots will produce the appropriate photosynthetic products as the fruit matures and the sink's capacity increases. The more fruit, the more it absorbs assimilates, and the more plant productivity.

Interestingly, in this research, the TFW-WP association was positive (Figure 2A). The WP (C_{WP} 0.34) also showed the highest-positive direct effect on yield under optimum conditions (Figure 2B). This means that crop production increases when water potential decreases until a certain level. The reduction in WP is required to extract and lift water from the soil to the plant body (Blum 2005). Blum (2017) also showed that genotypes with osmotic

adjustment strategies were identified to preserve low water potential under optimal conditions.

Under severe drought stress (35% FC), TFW was closely correlated with FN (0.54), FL (-0.53), and FD (0.74), leaf-related features (i) LN (-0.46); (ii) LAG (0.85); and (iii) CA (0.49), as well as the plant's dry weights: RPAR (0.68), SB (-0.46), LB (-0.66), and SR (-0.63) (Figure 2C). Additionally, LAG (C_{LAG} 0.48) together with the RPAR (C_{RPAR} 0.67) positively associated and highest-positive direct effect on yield under drought conditions (Figure 2D). The correlation between yield and yield-contributing characters under environmental stress has been well documented in chili under drought stress (Mahmood et al. 2021), tomato under shade stress (Ritonga et al. 2018), and soybean under acid soil (Kuswantoro 2017). Widuri et al. (2017) reported that leave sizes and numbers were indicators for chili under drought stress in the vegetative phase, while Malika et al. (2019) demonstrated the association of plant biomass with yield under drought in the generative phase in chili. As previously observed in wheat (Mohammadi 2018), tomatoes (Ritonga et al. 2018), and chilies (Widuri et al. 2017; Malika et al. 2019), changes in one or more yield-contributing traits are the primary cause of production variability. This situation explains that the total fluctuation in yield is principally governed by fluctuation in one or more yield-contributing characters.

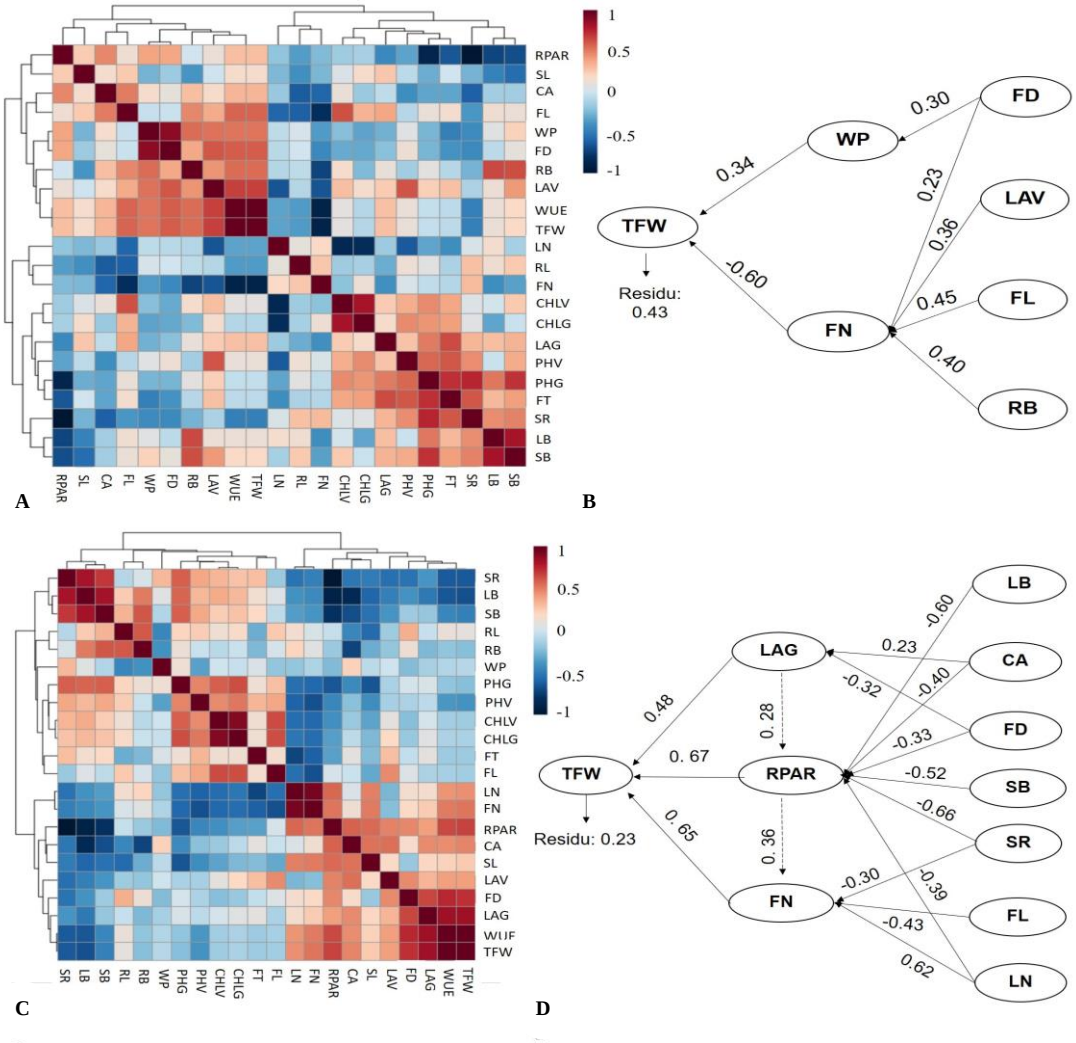

Figure 2. Pearson correlation and path analysis at the optimum and severe drought stress: A. correlation at optimum (80% FC); B. path coefficient under optimum (R square=0.8176; Residu=0.4271); C. correlation at severe drought stress (35% FC); D. path coefficient under severe drought stress (R square=0.9433; Residu=0.2381). Fruit characters were accumulated from all fruit harvested. RL: root length (cm), PHV: plant height at vegetative phase (cm); PHG: plant height at generative phase (cm); LN: leaves number; LAV: leaf area at vegetative phase (cm^2); LAG: leaf area at generative phase (cm^2); CHLV: leaf chlorophyll content at vegetative phase (SPAD); CHLG: leaf chlorophyll content at generative phase (SPAD); WP: leaf water potential (MPa); LB: leaves dry weight (g); SB: stem dry weight (g); RB: root dry weight (g); RPAR: root biomass proportion to total dry weight (%); SR: shoot-root ratio; CA: cell area (μm^2); SL: stomatal length (μm); FT: day to flowering (WAP); FN: number of fruit; FL: fruit length (cm); FD: fruit diameter (cm); WUEf: water use efficiency based on fruit fresh weight (gL^{-1}); TFW: total fruit weight per plant (g)

The FN had a direct negative effect on yield under optimal conditions (Figure 2B), but under severe drought conditions, it had a direct positive effect on yield (Figure 2D), but fruit length (FL) indirectly influenced yield. According to Aslani et al. (2020), fruit set has a significant impact on assimilate dispersion into the tomato fruit. Under stressful conditions, the genetic capacity for production is suppressed by environmental factors, causing plants to produce smaller or fewer fruit as an escape mechanism, whereas under optimum conditions the converse is true.

Regarding the correlation between FN and yield, Saleem et al. (2013) and Rosmaina et al. (2019b) reported a positive correlation between FN and yield and negative correlation between FL and yield under both optimum and drought stress conditions for tomato and curly chili, respectively. However, since the genotypes used in this study varied widely from big chili to bird-eye chili, it is challenging to determine if our findings confirm the previous conclusion. Additionally, the drought treatment in our experiment was carried out during the vegetative to generative phase, while Saleem et al. (2013) and Rosmaina et al. (2019b) simulated drought during the generative phase. Therefore, our findings complement their hypothesis.

We found that LAG, RPAR, and FN had higher total direct effects than indirect effects under severe drought stress. In addition, their heritability (97.09; 96.87; and 98.32) and genotypic variability (39.90; 25.63; 117.20) were high, suggesting that they are appropriate selection criteria to predict yield under severe drought stress. Previous reports have also mentioned FN as a selection criterion for chili under drought stress during the vegetative phase (Rosmaina et al. 2019b) and for tomato under shade stress (Ritonga et al. 2018). Meanwhile, Luo et al. (2019) summarize that root architecture is a good selection criterion for drought tolerance. It is assumed that plants adapt to cope with drought stress by changing leaf and root size, as well as the number of fruits per plant. The number of fruits per plant can be an appropriate selection criterion when chilies cannot be weighed immediately, while leaf area and root architecture can be used as selection criteria in the earlier growth phase to select for high-yielding and drought-tolerant genotype.

In summary, the study found that genetic factors had a greater influence on the morpho-physiological characteristics of chili genotypes compared to environmental factors. The broadest genotypic coefficient of variation (GCV) was recorded for yield (94.04), WUE per fruit fresh weight (88.88), fruit numbers (FN) (117.20), fruit length (FL) (46.58), and five vegetative organs at the generative phase. It showed that 35% FC is an appropriate selection environment for drought-tolerant traits in chili. Our experiment also revealed that LAG, RPAR, and FN, followed by FL, were essential selection criteria for effective yield improvement under severe drought stress. These findings complement previous information on selection criteria for drought tolerance at various growth phases.

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