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Induction of autotetraploid *Moringa* plant (*Moringa oleifera*) using oryzalin

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Abstract. *Ridwan, Witjaksono. 2020. Induction of autotetraploid* Moringa *plant (*Moringa oleifera*) using oryzalin. Biodiversitas 21: 4086-4093. Moringa* (*Moringa oleifera* Lam.) is a plant with high nutritional content and has been widely used as a vegetable and health food ingredient, but its utilization and productivity have not been optimal. Increasing plant productivity can be done by increasing biomass which can be obtained through increasing plant ploidy. This study aims at obtaining tetraploid *Moringa* by induction using oryzalin compound. *Moringa* germinating seeds were soaked in oryzalin water solutions for 1, 3, and 5 days with concentrations of 0 µM (control), 15 µM, 30 µM, 60 µM, and 120 µM with 10 seeds for each treatment. We have recovered two tetraploid *Moringa* plants and two chimeric plants of diploid and tetraploid genomes. Immersing germinating seeds for 1 day in 15 or 60 µM oryzalin concentration effectively induced autopolyploid in *Moringa*. Tetraploid plants exhibit typical morphological characteristics of a tetraploid, such as low stomata density, larger stomata size, and larger leaflet size compared to the diploid counterpart. The induced tetraploid plants have significantly increased protein, fat, and calcium content compared to the diploid counterpart emphasizing their values as functional food. This polyploidy induction method can be used to produce *Moringa* tetraploid lines from various accessions in the attempts of developing and selecting high yielding tetraploid *Moringa* cultivars.

Keywords: Drumstick tree, germinating seeds, *Moringa oleifera*, oryzalin, polyploidy, tetraploid

INTRODUCTION

Moringa that is also known as horseradish tree or drumstick tree (*Moringa oleifera* Lam.) is a fast-growing tree up to 10-12 m height (Parrota 2014) of the *Moringaceae* family and has a diploid chromosome of 28 (Samuel et al. 2015). The leaves are compound consisted of leaflets with 1.2-2.0 cm length and 0.6-1.0 cm width. *Moringa* grows from sea level to 2000 m above sea level and thrives on a variety of soil types (Raja et al. 2016). *Moringa* originated from the foothills of the Himalayas in North India, and spread to the tropical areas of Asia, Central to South America and Africa (Parrota 2014). In Indonesia, *Moringa* is spread in almost all southern regions, including Java, Madura, Lesser Sunda Islands such as Bali, Lombok to Alor (Riastiwi et al. 2018).

Moringa is consumed as a vegetable in some parts of Indonesia, i.e., eastern part. *Moringa* leaf vegetables can be a source of protein, minerals, and carbohydrates because of their high content of 22.75%, 13.02%, and 51.66%, respectively. Dried *Moringa* leaf has high nutrient density, which is 7 times of the vitamin C of oranges, 17 times of the calcium of milk, 10 times of the vitamin A of carrots, 9 times of the protein of yogurt, 15 times of the potassium of bananas, and 25 times of the iron of spinach (Koul and Chase 2015). Its antioxidant content is relatively high, especially the one from the flavonoid group (Muhammad et al. 2016; Edwinanto et al. 2018; Lin et al. 2018), even 3 times that of other vegetables (Pakade et al. 2013). Together with its content of sterol compounds and other diverse compounds, this plant can serve also as a functional food and a medicinal plant. In addition, the seed of this plant has high protein and fat content each about 32% (Melo et al. 2013) and has various industrial values such as feed, organic fertilizer (Parwata and Soemeinaboedhy 2018), and water purification (Bina et al. 2010; Beltran-Heredia et al. 2012, Winarno 2018). Although its benefits are very broad, *Moringa* is commonly planted as border plants, rarely in intensive cultivations. To encourage cultivation, it is necessary to develop superior varieties with high biomass production.

Efforts to develop such varieties could be done, alternatively, by inducing polyploidy, which is increasing plant chromosome set from diploid to polyploid. Plant ploidy levels affect biomass accumulation parabolically. For example, biomass accumulation increases from diploid to tetraploid and decreases at hexaploid and continues to decrease at octoploid in *Arabidopsis* (Corneillie et al. 2018). In Populus, the optimum ploidi level for biomass accumulation was demonstrated to be triploid (Liqin et al 2019). However, significant increase in biomass or leaf size from diploid to tetraploid has been demonstrated in many plants. Tetraploid bananas are bigger than triploid and diploid bananas (Poerba et al. 2014). Compared to the original diploid bananas, the induced polyploid bananas also show thicker leaves, larger leaf stems, and fatter fruits as shown in the tetraploid 'Mas Lumut' (Poerba et al. 2014), tetraploid 'Mas Madu' (Poerba et al. 2019a), tetraploid 'Rejang' (Poerba et al. 2017), 'Mas Jambe' autotetraploid (Poerba et al. 2018), 'Klutuk Sukun'

autotetraploid (Poerba et al. 2019b). Induced tetraploid teak also has leaves that are thicker than the diploid counterpart (Ridwan et al. 2018). Increased biomass due to increased ploidy has been widely reported (Ketsa et al. 2001; Ye et. al. 2010; Wang et. al. 2015).

Autopolyploidy could be induced in various ways, including treatment of shoot apices with antimitotic compounds that inhibit the formation of mitotic spindles such as oryzalin, colchicine, trifluralin (Gallone et al. 2014; Feng et al. 2017). Oryzalin has been reported to be effective in inducing polyploidy, for example in *Hylocereus megalanthus* (Tel-Zur et al. 2011), *Allium cepa* (Grzebelus and Adamus 2004), Hebe "Oratia Beauty" (Gallone et. al. 2014).

Induction of tetraploid Moringa plants by immersion of germinating seeds in oryzalin solution and characterization of morphological and nutritional traits of the resulting tetraploid plants are reported in this paper.

MATERIAL AND METHODS

Plant material was seeds collected and dried from *Moringa* plants grew in the garden of Research Center for Biology LIPI. The mature seeds were cleaned and their wings were removed and then soaked in sterile distilled water for 60 minutes. The seeds were then placed in Petri dishes lined with moist tissue paper until they germinated characterized by the growth of roots of 2-4 cm long. To induce polyploidy, the germinating seeds were then immersed in various concentrations of oryzalin at various times.

The experimental treatment consisted of 2 factors, i.e., the concentration of oryzalin (0, 15, 30, 60, and 120 µM), and the duration of immersion (1, 3, and 5 days). As many as 10 germinating seeds were immersed in each treatment solution. As much as 200 mL of treatment solutions were placed in a glass bottle with a capacity of 330 mL. The treatments were maintained in a room with temperature of 20-25ºC with relative humidity of 60-80% and with diffuse lighting. After the treatment period, the *Moringa* germinating seeds were planted in 5x10 cm plastic bag filled with growth media with composition of 2 topsoil, 1 charcoaled rice husk, and 1 compost. They were maintained under 55% shade. After 4 weeks, the seedlings were then transferred to growth medium in bigger plastic bags measuring 35 x 45 cm and placed in an open field for growth observation.

Phenotypic evaluation included: (i) plant growth including plant survival, plant height, stem diameter, number of compound leaves, and leaf area of a selected compound leaf; (ii) ploidy level of the resulting plants; and (iii) stomatal density and size. The plant survival is calculated from the number of seedlings that grow at the age of 1 and 4 weeks after planting (WAP) and then presented as percentage of survival. Observations on plant height, stem diameter, and number of compound leaves were carried out for all plants at 1.5 months after transferred to open land. For the leaf area observation, the identified polyploid plants and three randomly selected control plants at 2 months after being moved to the open land were sampled. The samples were 10 leaflets taken from the 4th compound leaf from apex. The leaflets were detached from the petioles and laid on a white paper for photographing. Photographs of the leaflets were analyzed with ImageJ 1.47v software to obtain leaf area.

Plant ploidy level was analyzed using CyFlow Ploidy Analyser (Partex, Germany). A sample of young leaflets measuring 1 cm^2 was suspended in 250 µL Cysteine PI Absolute then chopped into small pieces with a razor blade. The chopped leaflets were then filtered into a sample tube, then added with 1 mL of buffer solution containing Propidium Iodide and RNase) for relative DNA content reading. Leaflet samples from *Moringa* control plants were used as a diploid reference and their peak of relative value of DNA was set at a value of 200. Thus, the peak relative DNA content with a value of 400 indicates a tetraploid plant and the multiple peak values indicate the presence of multiple ploidy or chimera. Histograms that show peaks with values of 200 and 400 at the same time indicate chimeric plants with diploid and tetraploid chromosomes and called mixoploid. All plants grown from the treatments were sampled, while from the control treatment without oryzalin, only three plants of 1-day immersion were sampled.

Plants that had been identified as tetraploids and mixoploids were observed for their stomatal density and stomatal size and compared to diploid control to confirm ploidy. Leaflet samples were selected from the ones located at the base, middle, and upper part of each fully expanded compound leaf from every branch. The preparation for stomatal observation followed method developed by Haryanti (2010) with slight modification. Leaflet samples while still attached to the tree were smeared with transparent nail polisher (Revlon) at abaxial surface and allowed to dry. A piece of clear adhesive tape was pasted to overlay the dried smeared polisher. After a while, the tape was then removed bringing along the thin film of nail polisher with stomatal prints in it. The tape was then put on an objective glass for microscopic analysis. Observation of stomata density was done by counting the number of stomata manually with a light microscope at magnification 40 times and divided by visual field area (0.126 mm2), whereas observations of stomata size were carried out at 100 times magnification.

Proximate analyses, which comprised carbohydrate, fat, protein, calcium, and ash content were performed when the plants were 1.5 years old, with leaf samples from the 3rd to 7th from the apex. Fresh leaves were washed thoroughly with running water and then dried in an oven at a temperature of 55º for 3 days. The dried leaves were then blended to fine powder. The leaf powder was sent to a commercial laboratory (PT. Saraswanti Indo Genetech, Bogor) for the proximate analysis.

Statistical analysis was conducted with One-Way ANOVA using software SPSS for Windows version 16 at α=0.05. Differences among means were detected with DMRT (Duncan Multiple Range Test) at α=0.05. Additionally, some of the data were analyzed and presented as percentage, percentage increase, or means and their standard errors. The standard errors or referred to as deviations of samples (STDEV.S) were calculated with

Microsoft Excel and presented also as bars in histograms of the means. Percentage increase in proximate analysis values was calculated based on the value of the control diploid.

RESULTS AND DISCUSSION

Plant survival

The oryzalin induction treatments affected the survival of Moringa plants (Figure 1). Increase in oryzalin concentrations decreased the survival of the plants. The effect of oryzalin concentrations seemed to interact with immersion period. At 1-day immersion, the reduction in the percentage of survival dramatically occurred only at the highest oryzalin concentration of 120 µM. At 3-day immersion, the percentage of survival decreased by more than 50% at the concentration of 15 µM, and the plants did not grow at a concentration of 60 µM. At 5-day immersion, the effect of oryzalin concentrations was stronger in the reduction of survival percentage and leaving only 30% surviving plants, even at the lowest concentration of oryzalin of 15 µM. Most of the plants that managed to survive until the age of 1 week continued to grow and survive until 4 weeks, and only a few of them died, including some plants from control without oryzalin treatment.

In addition to reducing the percentage of germinating seeds that grew, oryzalin treatments also significantly affected plant growth features (Table 1). Oryzalin treatment decreased the average plant height. In the 1-day immersion treatment, even though the average plant height was lower than the control, but the high standard error value on the average treatment value showed a high variation on the plant height. Variable growth of stem diameter also decreased with oryzalin treatment, especially in immersion period of 3 and 5-days, whereas in immersion 1-day, only at 15 uM, the stem diameter was not significantly different from control. The average value of the number of plant leaves tended to increase with an increase in the concentration of oryzalin at 1-day immersion, but decreased at longer immersion time.

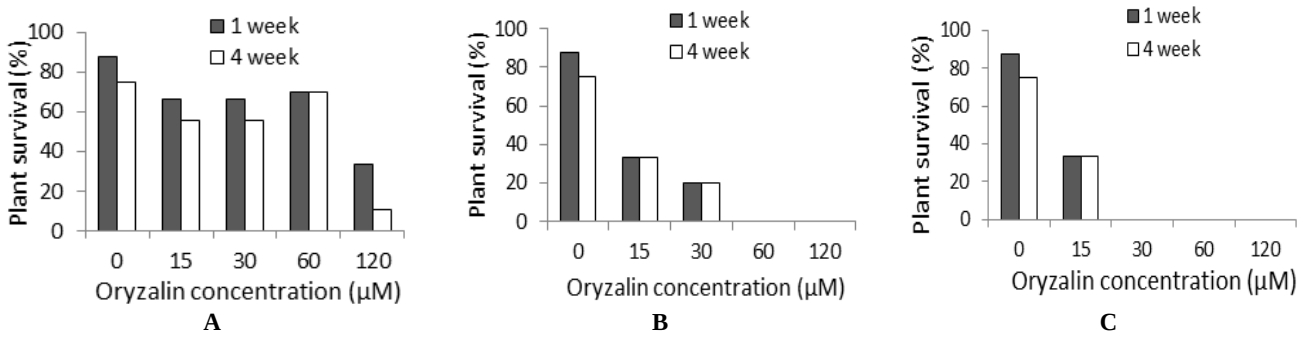

Figure 1. Effect of oryzalin concentrations of 0, 15, 30, 60, and 120 µM and immersion period of 1 day (A), 3 days (B), and 5 days (C) on plant survival percentage at 1 and 4 weeks after planting in an open field

Table 1. The effect of concentrations and immersion periods of germinating seeds in oryzalin solution on subsequent plant growth parameters 75 days after planting

Oryzalin treatments		Growth parameters		
Concentration (µM)	**Immersion period (days)**	**Height (cm)**	**Stem diameter (mm)**	**Leaf number (unit)**
0	1	58.50 ± 1.29 a	12.13 ± 0.76 a	8.75 ± 0.50 ab
15	1	48.2 ± 8.81 ab	11.24 ± 1.69 abc	12.00 ± 3.46 ab
30	1	38.25 ± 7.14 b	8.30 ± 0.67 d	12.50 ± 3.87 ab
60	1	42.83 ± 11.86 ab	9.27 ± 1.97 bcd	11.17 ± 3.25 ab
120	1	44.00 ± 0.00 ab	10.00 ± 0.00 abcd	8.00 ± 0.00 b
0	3	57.40 ± 14.08 a	12.26 ± 1.66 a	13.60 ± 3.05 a
15	3	34.00 ± 0.00 b	9.40 ± 0.00 bcd	8.00 ± 0.00 b
30	3	44.00 ± 0.00 ab	8.6 ± 0.00 d	8.00 ± 0.00 b
60	3	. . . . . no growth		
120	3	. . . . . no growth.		
0	5	59.33 ± 7.53 a	11.38 ± 0.88 ab	12.83 ±1.47 a
15	5	49.00 ± 1.41 ab	8.90 ± 0.85 cd	10.00 ± 1.41 ab
30	5	. . . . . no growth.		
60	5	. . . . . no growth.		
120	5	. . . . . no growth.		

Note: Data are presented as means ± standard error. Numbers in the same column followed by different letters are significant differences at p≤0.05 DMRT

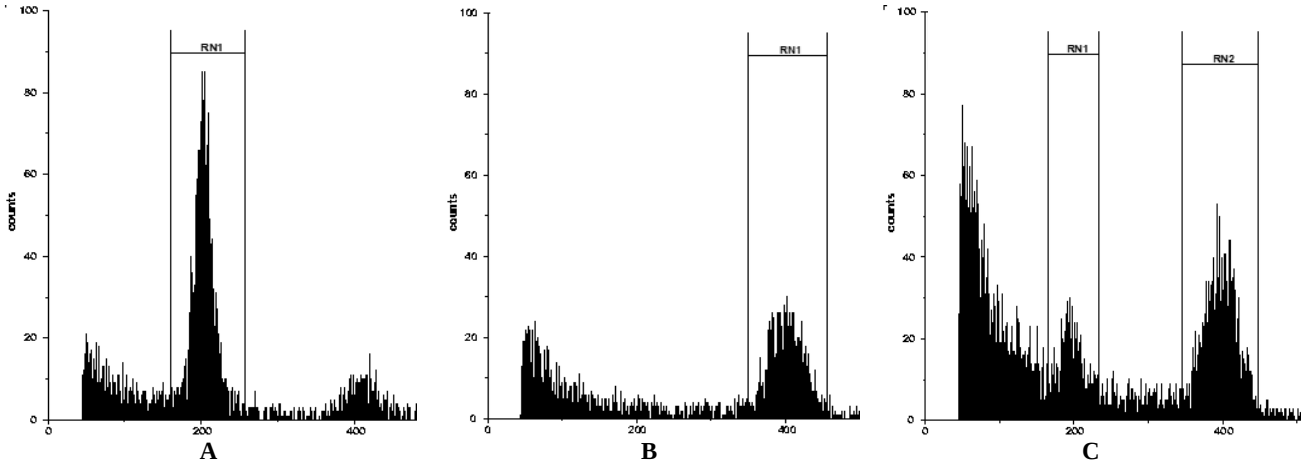

Figure 2. Graphs of flow cytometry analysis show peaks of control diploid *Moringa* plant and the treated plants indicating polyploidy. Peak of control diploid was set at channel 200 (A), peak at channel 400 (B) indicating a tetraploid plant and double peaks at channel 200 and 400 (C) indicating a mixoploid plant

Table 2. The effect of germinating seed treatment of concentration and immersion period in oryzalin solution on subsequent recovery of polyploid plant according to flow cytometer analysis, 2 months after planting

Oryzalin concentration (µM)	Immersion period (days)	Number of plants	Polyploid number	
			Mixoploid	Tetraploid
0	1	3	0	0
15	1	5	2	1
30	1	5	0	0
60	1	7	0	1
120	1	1	0	0
15	3	3	0	0
30	3	2	0	0
15	5	3	0	0

Ploidy analysis

The results of the flow cytometry analysis showed that the *Moringa* plants recovered from oryzalin treatment with various concentrations and duration of immersion could be grouped in 3 ploidy categories, i.e., diploid, mixoploid, and tetraploid. The plants from the control treatment were assumed to be diploid and their relative DNA contents were set at a peak of 200 (RN1) (Figure 2.A), while the tetraploid showed a peak value of 400 (RN2) as twice the diploid value (Figure 2.B), and mixoploid had both peaks, the peak at a value of 200 indicating diploid, and the peak at a value of 400 indicating tetraploid (Figure 2.C).

Flow cytometry analysis on *Moringa* plants recovered from the treatments of immersing germinating seeds for 1 day at a concentration of 15 µM resulted in 60% ploidy induction consisting of 40% mixoploid and 20% tetraploid. A tetraploid plant was also recovered with the same immersion duration treatment, but at a concentration of 60 µM. No polyploidy induction was obtained from other treatments (Table 2).

Stomatal density and size

The stomatal density of the diploid control *Moringa* plants reached more than 300 mm^{-2} uniformly in all the sampled branches, whereas the tetraploid plants had stomatal density only around 200 mm^{-2} and were evenly distributed in the 2-4 branches observed (Figure 3). However, in mixoploid plants, the density of stomata varied between branches with low density similar to the density of tetraploid plants, and branches with high density similar to diploid plants. Therefore, ploidy differences in mixoploid plants occurred in different branches.

Measurement of stomatal length and width confirmed that tetraploid plants had stomatal length and width that were significantly longer than those of diploid plants, whereas mixoploid plants showed stomatal length and width between the two ploidies (Figure 4).

Leaf area

The area of leaflets increased significantly with increase in plant ploidy with an increment of about 300% from diploid counterpart to tetraploids. However, leaf area variations occurred in mixoploid plants, both of which were characterized by big standard error. Leaf area of one of the mixoploids, number 15.1.1, was not significantly different from that of the diploid plants, but with greater variation in data. This showed that number 15.1.1 had more variability in leaf area than the diploid counterpart. Mixoploid plant of 15.1.2 showed a high diversity of leaflet areas with significantly higher values than other mixoploids and similar to leaf area of the tetraploid plants (Figure 5).

Nutrient content

Proximate analysis of diploid and the recovered polyploid plants showed differences in percent carbohydrate, protein, fat, ash, and calcium content (Table 3). Increased in ploidy increased leaf protein levels up to 20%, but decreased carbohydrate levels by 12%. The

increase in leaf protein content in tetraploid plants seemed to be offsetted by a decrease in carbohydrate content. This symptom was evident in a mixoploid plant of 15.1.1 which showed an increase in protein of up to 11% but was also compensated by a decrease in carbohydrates by 11%. Fat content also increased with an increase in ploidy, even up to 34% in a mixoploid, except in 1 tetraploid genotype whose increases were not significantly different. Ash content in one of the tetraploid genotypes of 60.1.4 significantly increased by 20% compared to diploid control. This increase was also shown by an increase in leaf calcium levels significantly as well. The proximate value of mixoploid plants tended to resemble the proximate value of tetraploid plants more than diploid plants, except for the calcium content of one mixoploid plant which was lower than the diploid plants.

Discussion

Solid tetraploid *Moringa* plants have been recovered by immersing germinating seeds in oryzalin solution for 1 day with a concentration of 15 and 60 µM along with mixoploid plants and diploids. Induction of tetraploid *Moringa* shoots in tissue culture using colchicine has been reported in an abstract with no detailed information (Zhang et al. 2007). Polyploidy induction with shoot tissue often produced mixoploid and therefore requires chimera separation, for example through repeated subcultures up to 3-5 passages to obtain intact polyploid plants, such as in bananas (Van Duren et al. 1996, Poerba et al. 2014, 2017, 2018, 2019a, 2019b). Efficient use of germinating seeds as target tissue for polyploidy induction without too much effort to separate chimera has also been demonstrated in oil palm (Madon et al. 2005), *Watsonia* species from South Africa (Thompson et al. 2010), *Phlox drummondii* (Tiwari and Mishra 2012), *Rhodomyrtus tomentosa* (Normasiwi and Nurlaeni 2014), water spinach *Ipomea aquatica* (Rahmi et al. 2019), and *Lilium rosthornii* plant (Wang et al. 2020).

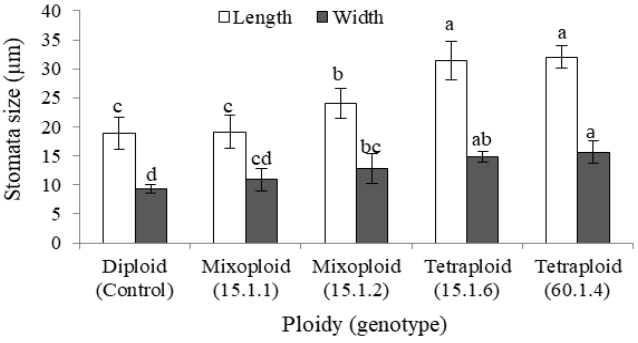

Figure 4. Comparison of the average length and width of the stomata of the diploid control *Moringa*, mixoploid plants of 15.1.1 and 15.1.2, and tetraploid of 15.1.6 and 60.1.4. Different letters indicate significant differences at ≤0.05 DMRT

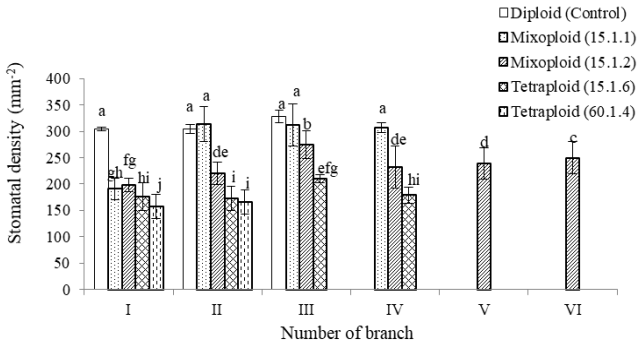

Figure 3. Comparison of the average of the stomatal density of leaflets grew from different branches of the diploid control Moringa, mixoploid plants of 15.1.1 and 15.1.2, and tetraploids of 15.1.6 and 60.1.4. Different letters indicate significant differences at ≤0.05 DMRT.

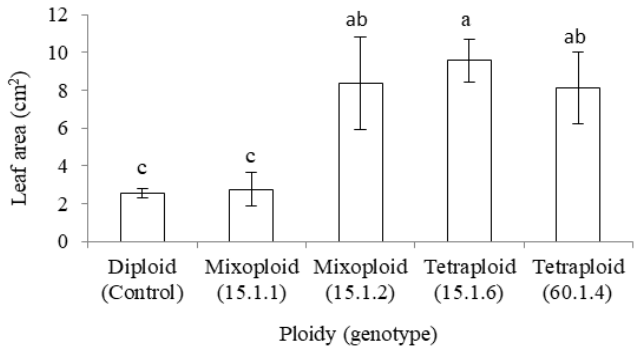

Figure 5. Comparison of leaflet area of diploid, mixoploid, and tetraploid *Moringa* plants. Different letters indicate significant differences at ≤0.05 DMRT

Table 3. Proximate analysis of *Moringa* leaf of control diploid plants, and the induced polyploidy plants

Genotype (ploidy)	Carbohydrate		Protein		Total fat		Ash		Calcium	
	Content (%)	Increase (%)	Content (%)	Increase (%)	Content (%)	Increase (%)	Content (%)	Increase (%)	Content (mg/100 g)	Increase (%)
Control (diploid)	51.9±0.4 a		27.1±0.2 b		5.7±0.2 a		8.1±0.4 b		1833.8±380.0 a	
15.1.1 (mixoploid)	46.2±1.0 b	-11	30.0±1.0 ab	11	7.6±0.5 a	34	9.6±0.3 a	19	2203.8± 42.4 a	20
15.1.2 (mixoploid	43.1±2.4 b	-17	33.3±1.7 a	23	7.1±0.4 a	25	9.0±0.3 ab	12	1776.6±211.4 a	-3
15.1.6 (tetraploid)	45.6±1.8 b	-12	32.8±1.5 a	21	6.8±0.3 a	20	9.0±0.2 ab	12	1967.5±182.9 a	7
60.1.4 (tetraploid)	45.6±1.5 b	-12	31.3±1.7 ab	16	6.5±0.7 a	13	9.6±0.8 a	19	2412.8±122.7 a	31

Data are presented as mean and standard error. Numbers followed by different letters are significantly different at ≤0.05 DMRT. Percentage increases were calculated based on the value of the control diploid.

Moringa germinating seeds did not tolerate immersion duration of 3-5 days, especially at the concentration of oryzalin more than 30-60 µM. Determination of the duration and dose of exposure to the compounds inhibiting the formation of microtubule compounds is the key to the success of polyploidy induction. The optimal soaking time in oryzalin solution differs with different concentrations, but ranges from one day for *Lilium rosthornii* with a concentration of 0.01% (34.6 µM) (Wang et al. 2020), two days for *Hylocereus megalanthus* with a concentration of 0.0005% (0.7 µM) (Tel-Zur et al. 2011) and Hebe "Oratia Beauty" with a concentration of 289 µM (Gallone et. al. 2014), three days for *Allium cepa* plants with a concentration of 50 µM (Grzebelus and Adamus 2004).

The effective concentration of oryzalin of 15 µM for the induction *Moringa* autotetraploid is considered a low concentration. Oryzalin is used for induction of polyploidy with very wide concentration range depending on the target plant species, from a very low one at 0.7 uM for *Hylocereus megalanthus* (Tel-Zur et al. 2011) to the highest at a concentration of 289 µM for hebe 'Oratia plants Beauty' (Gallone et al. 2014). In most plants, the concentration of oryzalin at tens of micromolar intervals proved effective for the induction of polyploidy, for example in *Aframomum corrorima* plants at a concentration of 10 µM (Wannakrairoj & Tefera 2013), *Lychnis* spp. at a concentration of 10 mg L^{-1} (28.9 µM) (Nonaka et al. 2011), cayenne pepper at a concentration of 10-30 mg L^{-1} (28.9-86.6 µM) (Pliankong et al. 2017), orchid plants at a concentration of 57,7 µM (Miguel and Leonhardt 2011), on bananas at a concentration of 15-60 µM (Van Duren et al. 1996; Poerba et al. 2014, 2017, 2018, 2019a, 2019b).

Induced tetraploid *Moringa* plants show morphological characteristics that are typical of tetraploid and are different from diploid plants, which among others have lower stomatal density but larger stomata size. These characters are in accordance with the results of tetraploid induction experiments on water spinach (Rahmi et al. 2019), teak (Ridwan et al. 2018), bananas (Poerba et al. 2014, 2017, 2018, 2019a, 2019b). These properties could be used to identify polyploid plants in many plants when flow cytometers are not available, for example in cotton plants (Wongpiasatid et al. 2005), some plants of the *Orchidaceae* such as the orchid *Dendrobium*, *Epidendrum*, *Odontioda*, and *Phalaenopsis* (Miguel and Leonhardt 2011), cactus (Tel-Zur et al. 2011), *Phlox drummondii* (Tiwari and Mishra 2012), and cayenne pepper plants (Pliankong et al. 2017).

Tetraploid *Moringa* plants obtained showed a larger leaf size than diploid plants. The size of the vegetative part of polyploid plants which is larger than diploid origin has been shown in various plants, including *Swainsona formosa* (Zulkarnain 2004), crape myrtle flowers (Ye et al. 2010), bananas (Poerba et al. 2014, 2017,2018, 2019a, 2019b), pear (Wang et al. 2015), daylily (Podwyszynska et al. 2015) and teak seedlings (Ridwan et al. 2018). For this reason, the tetraploid induction in *Moringa* is intended to increase biomass which ultimately increases its productivity. This evidence has not been obtained until the tetraploid *Moringa* plants can be propagated in sufficient quantities and tested agronomically. However, proximate analysis demonstrated that tetraploid plants have higher levels of protein, fat, and minerals than diploid plants indicates the potential of induced tetraploid *Moringa* plants as functional food. *Moringa* has been traded as flour and is used as a mixture for making noodles, cakes, and even cosmetic products. *Moringa* leaf flour with higher protein, fat and mineral content could be an alternative solution for national nutritional problems such as stunting caused by undernourishment (Uauy et al. 2015; Sari et al. 2016; Sulistiani and Yanti 2016).

The conclusion is that induction of polyploidy in *Moringa* plant could be done by immersing germinating seeds with 2-4 cm roots in 15-60 µM oryzalin solutions for one day. A total of two tetraploid plants and two mixoploid plants have been recovered in accordance with the results of the flow cytometer analysis confirmed with low stomatal density and large stomatal size. Tetraploid plants exhibit typical polyploid characteristics, i.e., larger leaflets than the original diploid plant. Tetraploid plants also show increased levels of protein, fat, and minerals but decreased carbohydrate levels. These characteristics conform to functional food criteria and could be used as an alternative to mitigate one of the national nutritional problems such as stunting. The effectiveness of the polyploidy induction described in this paper could be recommended as a method to produce tetraploid *Moringa* lines from many seeds from various varieties or genotypes. With selections and agronomic evaluations, it is expected that superior tetraploid *Moringa* cultivars with high productivities and nutritional contents could be developed.

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