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Penapisan Secara *In Vitro* Kentang (*Solanum Tuberosum* L) Toleran Kekeringan Koleksi IPB

(In Vitro Screening of Drought Tolerant Potatoes (*Solanum tuberosum* L) of IPB Collections)

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ABSTRACT

In supporting the replacement of potato varieties with the varieties that are adaptive to drought, effective and fast methods are needed. One of which is through in vitro screening of drought-tolerant genotypes. The aims of this research were to determine the appropriate concentration of sorbitol as a critical limit in the in vitro screening process of drought-tolerant genotypes, as well as to determine vegetative characteristics that could be used as indicators of in vitro selection and also to obtain in vitro drought-tolerant genotypes. To achieve these objectives, we used single-nodal explants of seven genotypes cultured for six weeks on MS media added with four levels of sorbitol concentration. Of the four treatments, sorbitol level that could distinguish susceptible and tolerant plants was 0.2 M sorbitol and at this concentration, the characteristics that showed significant interactions were plant height and root length, so that both characteristics could be used as selection indicators for the analysis of tolerance tests for the seven genotypes. The results of tolerance test using the analysis of relative decline and stress-susceptibility index (SSI) showed that PKHT4 and PKHT6 were the genotypes that were tolerant in vitro.

Keywords: drought tolerant*, in vitro, Solanum tuberosum*, sorbitol

ABSTRAK

Dalam mendukung pergantian varietas kentang dengan yang adaptif terhadap kekeringan diperlukan metode yang efektif dan cepat, salah satunya melalui penapisan genotipe toleran kekeringan secara *in* vitro. Untuk itu tujuan penelitian ini adalah menentukan konsentrasi sorbitol yang tepat sebagai batas kritis dalam proses penapisan genotipe toleran kekeringan secara *in vitro*, serta mendapatkan karakter vegetatif yang dapat dijadikan sebagai indikator seleksi secara *in vitro* dan juga mendapatkan genotipe toleran kekeringan secara *in vitro*. Untuk mecapai tujuan penelitian, digunakan eksplan buku tunggal dari tujuh genotipe yang dikulturkan selama enam minggu pada media MS yang ditambahkan empat level konsentrasi sorbitol. Dari keempat level sorbitol yang dapat membedakan tanaman peka dan toleran adalah sorbitol 0,2 M dan pada konsentrasi ini karakater yang memiliki interaksi nyata adalah tinggi tanaman dan panjang akar, sehingga kedua karakter tersebut dapat dijadikan indikator seleksi untuk analisis uji toleransi ketujuh genotipe. Hasil uji toleransi dengan menggunakan analisis penurunan relatif dan *stress susceptibility index* (SSI) menunjukkan PKHT4 dan PKHT6 adalah genotipe toleran secara *in vitro*.

Kata kunci: genotypes, *in vitro*, *Solanum tuberosum,* sorbitol, toleran kekeringan

INTRODUCTION

Potato cultivar that is tolerant to drought is an urgent need because, as stated by Boguszewska-Mańkowska *et al.* (2020), prolonged drought stress in the areas of potato-plants growing can cause a rapid and drastic decline in the production during the 21st century, coupled with the increased demand for water and unavailability of water (Tuberosa 2012). Therefore, understanding of drought tolerance or drought avoidance by plants is very important. Potato plants are very sensitive to drought stress because they have shallow roots, and the growths of leaf and tuber are sensitive to mild drought-stress, especially during the phase of tuber initiation (Obidiegwu *et al*. 2015).

Climate change causes longer dry season and drought in many potato cultivation regions so that the substitution of potato varieties with varieties that are adaptive to drought must be performed faster and this can be fulfilled through a rapid breeding cycle (Austin 2011; Tardieu 2012). However, in developing countries such as Indonesia, the system of seed replacement is still slow (Monneveux *et al.* 2014), and according to Atlin *et al.* (2017), a faster system of seed replacement with tolerant varieties according to climate change must be improved. The rapid breeding cycle must be supported by the effective selection methods that are

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easy and fast. To determine an effective selection method, proven screening methods and selection indicators are needed.

In the selection process of drought-tolerant plants, plant materials as donors of drought-tolerant characteristics are needed. Therefore, a collection of potatoes in effort to increase the diversity is required. Potato collections at IPB University, Indonesia have been established for a long time with the aim of providing superior potato seeds through a series of researches (Neni *et al.* 2018). The IPB collection of promising potato clones with high and stable production includes PKHT9 and PKHT10 clones which are suitable to be used as vegetable potatoes, and PKHT4 and PKHT6 which are suitable to be processed into potato chips (Zulkarnain *et al.* 2017). From observations in the field, these clone collections also have a potential of drought tolerant. This research was carried out to examine the tolerance of potato clones and to determine the appropriate method for screening the drought-tolerant genotypes.

The in vitro screening method is one of the initial screening methods that can be performed quickly in large quantities to obtain drought-tolerant potatoes. The agents for in vitro drought selection that are often used are PEG and sorbitol. Research by Anithakumari *et al.* (2011) screened 94 drought-tolerant genotypes in vitro using PEG as a selection agent. In addition to PEG, research by Gopal & Iwama (2007) also used sorbitol to examine morphological responses. Albiski *et al.* (2012); Bündig *et al.* (2016) also used sorbitol as a drought-selection agent to examine physiological responses in the form of proline and osmotic adjustment. In in vitro drought-tolerant screening processes, the critical concentration limits of the selection agent used must be determined. At this concentration, the plantlet still has a life capacity of 40−60% by calculating the relative decline, i.e., the difference of each characteristic at each concentration of sorbitol from the control (Bündig *et al.* 2016). To help determine the critical limit of the selection-agent concentration, the orthogonal polynomial analysis can be used since this analysis can explain the form of characteristic responses. Furthermore, the concentration used as a critical limit will determine the characteristics that can be used as indicators of in vitro selection, and based on these selected characteristics, drought tolerance of the tested genotypes will be evaluated by using analysis of relative decline and stress-susceptibility index (SSI). Therefore, the aim of this study was to perform the in vitro screening process of drought-tolerant potato plants.

MATERIALS AND METHODS

Site and time of research

The experiments were carried out in the laboratory of the Center for Tropical Horticulture Studies (CTHS), IPB University, Bogor, from February 2018 to August 2019.

Research Procedure

- **Genotypes used**

The experiment used seven potato genotypes consisting of five in vitro collections of CTHS laboratory (PKHT3, PKHT4, PKHT6, PKHT9, and PKHT10) and two commercial genotypes (Granola and Atlantic) as standard for comparison. The experiment was started with plant propagation. Propagation of plants was carried out using the basic media Murashige Skoog (MS), 30 g.L^{-1} sucrose and 7 g.L^{-1} agar compactor. The culture of plant propagation was incubated for three weeks before being transferred to the treatment media using shoot cuttings.

- **In Vitro drought-screening media**

This experiment used sorbitol as a drought-induction agent on induction media of potato micro-cuttings. The composition of in vitro screening media was adapted from Gopal & Iwama (2007) consisted MS + sucrose 30 g.L^{-1} + agar 7 g.L^{-1} + sorbitol (0.1, 0.2, 0.3, 0.4 M) in which sorbitol weight and water potential are presented in Table 1. The experiment was started with plant propagation. Propagation of plants was carried out using the basic media of Murashige Skoog (MS), 30 g.L^{-1} sucrose, and 7 g.L^{-1} agar compactor. The culture of plant propagation was incubated for three weeks before being transferred to the treatment media using shoot cuttings. Before transferring the media into the culture bottles (size of 150 mm x 25 mm), the pH of the media were adjusted to 5.7 ± 1.0. In vitro potato-shoot buds were transferred to the treatment media and were cultured at 19.7-20 °C for six weeks with 24 hour daily irradiation using white fluorescent lamps which produced 100 µmol m^{-2}s^{-1}.

Observation

Six weeks after planting (WAP), the plantlets were removed from the bottle, and each plantlet per genotype was cut from the root and plantlet height was measured as the main stem length from the base to the tip. They were weighed as a fresh weight and after drying it was weighed as a dry weight. The roots were washed to release the sticky agar media and then measured for the root length and weighed as the root

Table 1 Sorbitol concentrations and water potentials

Sorbitol concentration (M)	Sorbitol Weight (g)	Water Potential (Mpa)
Control	-	- 0.80
0.1	18.217	- 1.00
0.2	36.434	- 1.35
0.3	54.651	- 1.70
0.4	72.868	- 2.50

fresh-weight, and after being dried then it was weighed as the root dry-weight. For dry-weight determination, the plantlet canopies or aerial parts and the roots were dried using an oven with a temperature of 70°C for 48 hours.

Data analysis

Data processing used SAS 9.1.3 software for the analysis of variance (ANOVA), and the Polynomial Orthogonal analysis using Star software was performed to determine the response patterns. This study used a completely randomized design (CRD) consisting of drought treatments with five levels (T0: without sorbitol, T1: 0.1 M, T2: 0.2 M, T3: 0.3 M, T4: 0.4 M sorbitol) and three replications so that the total of experimental unit was 105. The relative decline (RD) analysis was performed to determine the critical limit of sorbitol concentration (Bündig *et al*. 2016).

$$RD\ \frac{PO-Ps}{PO}\ X\ 100\% \quad (1)$$

Description:
P0 = Characteristic observed in the control
Ps = Characteristic observed in sorbitol concentrations

Determination of the susceptible and tolerant potato genotypes was carried out using the stress susceptibility index (SSI) test according to Fischerab & Maurer (1978):

$$SSI = \frac{1-\left(\frac{Ps}{PO}\right)}{1-\left(\frac{mean\ PS}{mean\ PO}\right)}\ X\ 100\% \quad (2)$$

Description:
Ps = Characteristic observed in sorbitol concentrations
P0 = Characteristic observed in the control
Mean Ps = Average of all genotypes in sorbitol
Mean P0 = Average of all genotypes in the control

RESULTS AND DISCUSSION

Determination of the Critical Limit of Sorbitol Concentration as a Selection Agent

ANOVA results (Table 2) showed that genotypes and sorbitol gave significant differences on all characteristics. The interaction between genotypes and sorbitol was also significantly different in all characteristics, except that the canopy dry weight was not significantly different. This means that sorbitol can be used as an in vitro selection agent. Furthermore, determination of the appropriate level of sorbitol for drought-tolerance screening media was determined by calculating the value of Relative Decline (RD). The results of the relative decline calculation indicated a decrease in growth in all genotypes at the four sorbitol concentration levels with various degrees of decline. At sorbitol concentration of 0.1 M, all canopy characteristics (Table 3) and root characteristics (Table 4) experienced a decrease in growth below 50% compared to the controls, except for the canopy fresh-weight that reached 50.85%, where the lowest growth decrease in root-length characteristic was only 18.79%. Based on the ANOVA test between sorbitol concentration of 0.1 M and the control, root length, canopy dry-weight and root fresh-weight on sorbitol treatment were not significantly different from the control. In contrast, plant height, root fresh-weight, and root dry-weight on sorbitol treatments were different from the control; however, in all canopy and root characteristics, there was no interaction between genotypes and sorbitol treatments.

Drought-like effect or osmotic stress due to the addition of sorbitol or PEG in in vitro media has been shown to inhibit plantlet growth (Anithakumari *et al.*, 2011, Albiski *et al.*, 2012, and Bündig *et al*., 2016). However, sorbitol decreased more the water potential of in vitro media compared to PEG, in which the higher the concentration of sorbitol, the higher the decease of water potential of the media. Low water potential due to the addition of sorbitol into the in vitro media (Table 1) could induce drought stress because the roots have difficulty in absorbing water into the plant tissue. The results of this study also showed that sorbitol could induce drought stress in vitro as was evidenced by the results of ANOVA (Table 2). There were significant interactions between genotypes and sorbitol treatments in all characteristics, except canopy dry-weight. These results were similar to those of Gelmesa *et al.* (2017), Nasiruddin & Islam (2018), Aliche *et al.* (2018) that there were interaction effects between genotypes and sorbitol on–canopy fresh-weight, root length, and root dry-weight.

At 0.2 M sorbitol concentration (Tables 3 and 4), the lowest relative-decline value occurred in the canopy

Table 2 Effects of sorbitol concentration levels in nine potato genotypes on canopy and root morphological characteristics in vitro

Sk	Db	Characteristic					
		PH	CFW	CDW	RL	RFW	RDW
Genotype	6	0.34**	0.00039**	0.0000047**	1.897**	0.00020**	0.0000054**
Sorbitol	4	15.47**	0.0065**	0.000025**	23.868**	0.0009**	0.0000056**
G x S	24	0.42**	0.00027**	0.0000017 ns	0.871**	0.000053*	0.0000015**
Random	70	0.076	0.00012	0.0000015	0.19	0.000029	0.000000015
CV		11.50	1.50	0.17	18.48	0.75	0.005

Description: * Significant at α = 0.05, ** Very Significant at α = 0.01, PH: plant height; CFW: canopy fresh-weight; CDW: canopy dry-weight; RL: root length; RFW: root fresh-weight; RDW: root dry-weight. ns: nonsignificant

Table 3 Relative decline and analysis of variance of each sorbitol concentration and the control in the canopy characteristics of seven potato genotypes

Genotype	Plant Height				Canopy Fresh-Weight				Canopy Dry-Weight			
	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
PKHT3	33.91	51.75	76.01	79.90	43.84	63.50	79.46	83.67	30.65	24.03	57.47	62.5
PKHT4	15.29	26.25	62.90	73.86	25.12	13.35	49.86	71.22	39.50	0	38.56	51.18
PKHT6	2.96	29.04	58.25	66.14	62.89	65.62	72.58	80.73	57.69	65.38	70.51	76.92
PKHT9	39.78	46.43	76.03	86.80	54.21	60.56	63.80	86.92	0	10.59	42.25	53.36
PKHT10	37.26	49.76	72.84	100	56.65	73.38	82.97	100	34.43	48.52	50	100
GRANOLA	37.09	56.40	68.40	100	58.28	61.44	68.25	100	39.59	41.86	61.34	100
ATLANTIC	41.45	67.92	79.39	100	54.93	77.14	90.94	100	43.24	43.69	60.30	100
AVERAGE	29.67	46.79	70.55	86.67	50.85	59.28	72.55	88.93	31.02	33.41	54.35	77.71
Genotype	**	**	**	**	**	ns	**	*	*	ns	ns	ns
Sorbitol	**	**	**	**	**	**	**	**	ns	ns	**	**
GXS	ns	*	**	**	Ns	ns	*	**	ns	ns	ns	ns
CV	8.97	9.508	10.480	13.69	0.184	1.508	1.140	1.121	0.184	1.182	0.181	0.183

Description * Significant at α = 0.05, ** Very Significant at α = 0.01, ns: nonsignificant, PH: plant height; CFW: canopy fresh-weight; CDW: canopy dry-weight; RL: root length; RFW: root fresh-weight; RDW: root dry-weight, T1: sorbitol 0.1 M, T2: sorbitol 0.2 M, T3: sorbitol 0.3 M, T4: sorbitol 0.4 M.

Table 4 Relative decline and analysis of variance of each sorbitol concentration and the control in the root characteristics of seven potato genotypes

Genotype	Root Length				Root Fresh-Weight				Root Dry-Weight			
	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
PKHT3	15.40	31.10	71.57	100	56.17	70.28	96.10	100	75.00	88.25	88.80	100
PKHT4	20.74	32.81	47.31	90.86	45.72	80.74	80.92	98.29	58.63	67.54	81.28	99.18
PKHT6	13.99	35.26	46.60	100	1.81	55.02	73.90	100	8.33	22.22	66.67	100
PKHT9	27.10	61.63	73.71	91.42	5.15	83.09	96.22	98.45	16.67	56.67	76.17	91.63
PKHT10	21	57.59	80.60	100	22.75	50.49	82.35	100	43.06	68.78	75.78	100
Granola	19.64	38.84	57.41	100	68.49	79.29	93.52	100	99.25	49.61	79.55	100
Atlantic	13.68	42.11	100	100	24.26	61.70	100	100	3.17	53.87	100	100
Average	18.79	42.76	68.17	97.47	32.05	68.66	89.00	99.54	43.44	58.13	81.18	98.69
Genotype	**	**	**	**	*	ns	ns	ns	ns	ns	ns	ns
Sorbitol	ns	*	**	**	ns	ns	**	**	*	ns	**	**
GXS	ns	*	Ns	**	ns	ns	ns	ns	ns	ns	ns	ns
CV	15.25	26.78	24.52	21.43	0.99	2.51	0.921	0.92	0.109	0.104	0.109	0.108

Description: * Significant at α = 0.05, ** Very significant at α = 0.01, ns: nonsignificant, PH: plant height; CFW: canopy fresh-weight; CDW: canopy dry-weight; RL: root length; RFW: root fresh-weight; RDW: root dry-weight, T1: sorbitol 0.1 M, T2: sorbitol 0.2 M, T3: sorbitol 0.3 M, T4: sorbitol 0.4 M.

dry-weight (33.41%) and the highest relative-decline value occurred in the root fresh-weight (68.66%). The results of ANOVA between 0.2 M concentration and the control showed that the root length was significantly different, the plant height was very significantly different, and there were interactions between genotypes and sorbitol concentration. There were no significant interactions effects between genotypes and sorbitol concentrations on canopy fresh-weight, canopy dry-weight, root fresh-weight, and root dry-weight.

At 0.3 M and 0.4 M sorbitol concentrations (Table 3 and 4), growth decreases were above 70%, except for canopy dry-weight (54.35%) and root length (68.17%). These results showed that the selection agent at these two concentrations cannot be used because plants have very high inhibition of growth at both concentrations. Of the four tested levels of sorbitol, the concentration that can be used as a critical limit in the in vitro screening process of drought-tolerant potato plants was 0.2 M sorbitol. This selection was based on the RD values of most characteristics that were below 50% and the results of ANOVA between each concentration and the control on the canopy and root characteristics showed that plant height and root length were significantly affected by genotype and sorbitol interactions. When compared to 0.1 M concentration, the RD value was also below 50%, but the results of ANOVA showed that all characteristics were not significantly affected by the genotype and sorbitol interactions. The sorbitol concentrations of 0.3 M and 0.4 M showed RD values above 50−98.69% in the root dry-weight at 0.4 M sorbitol. If the decrease reaches 60%, the plant's response to drought cannot be evaluated properly because the abilities of plants to grow are very low when exposed to stressed condition. The decreased growth that occurred in this study was due to the drought-like effect caused by sorbitol addition. According to Aliche *et al.* (2020), drought caused the decreased water content of cells and tissue causing a turgor pressure to decrease and consequently inhibits cell elongation. Therefore, 0.2 M sorbitol concentration can become a selection agent for in vitro screening selection of drought tolerance because at this concentration, there were characteristics that can distinguish genotypes. These results are in agreement with those of Albiski *et al.* (2012); Obidiegwu *et al. (*2015); Gelmesa *et al.* (2017).

Furthermore, based on the results of ANOVA on 0.2 M sorbitol concentration and the control (Table 3 and 4), there were significant interactions effects between genotypes and sorbitol treatments on the plant height and root length. Therefore, both characteristics can be used as indicators of in vitro selection. The results of Bündig *et al*. (2016) also showed that the characteristic of canopy dry-weight has a relative decline value below 50%, but Bundig *et al*. (2016) cannot describe how the interaction between genotypes and sorbitol in media without sorbitol and media containing 0.2 M sorbitol. By understanding whether there is a interaction between genotype and sorbitol (Gelmesa *et al.* 2017; Nasiruddin & Islam 2018), the more valid characteristics can be determined as an indicator of selection.

Forms of Response of Canopy and Root Characteristics to Sorbitol

The response of plants to various levels of stress has the specific forms or response patterns. The forms of response of seven genotypes in this study showed that plant height, canopy dry-weight, and root dry-weight had a linear response pattern, while root length, canopy fresh-weight, and root fresh-weight had a quadratic response pattern (Table 5). Although the response patterns were different, both root and canopy characteristics showed the negative response patterns (Figure 1), which means that the higher sorbitol concentration generated the lower canopy and root characteristic values.

Plant height, canopy dry-weight, and root dry-weight (Figure 1a, 1c, 1f) had linear response patterns due to decreased growth from one level of sorbitol to another level of sorbitol. The decline value was almost the same so that the response forms of the two characteristics showed almost the same slope. Root dry-weight had a greater slope due to a very large decrease above 50% at the beginning of the concentration (0.1 M), but a decrease in the next level to 0.2 M and to 0.3 M showed the same value. This condition caused the form of response was significant in the linear forms of canopy fresh-weight, root length, and root fresh-weight (Figure 1b, 1d, 1e) even though there were quadratic response patterns due to a large decrease in the value of the control by increasing sorbitol concentrations, and its decreasing did not have the same values from one concentration to the other. However, the form of the decrease pattern in the root length at 0.2 M sorbitol concentration decreased below 50%, while in the canopy fresh-weight and the root fresh-weight, a sharp decrease had occurred at 0.1 M sorbitol concentration.

The response forms of the two characteristics chosen as indicators for selection were different, in which the plant height was linear (Figure 1a), while the root length was quadratic (Figure 1d). From the figures, it can be observed that plant height has decreased from level to level with the same value but the decreased root length at the beginning of 0.1 M sorbitol concentration experienced a small decrease and then the sorbitol decreased more with higher decrease value. This condition is in accordance with result reported by Dahal *et al.* (2019) that root response is direct responses of plants to drought and osmotic stress, in an effort to improve the ability to obtain water. This means plants having lower root growth or having lower RD values will be better able to obtain water, and this is a tolerance mechanism in vitro (Bündig *et al.* 2016). Root characteristic is very important in the development of drought-resistant idiotypes especially root length (Khan *et al.* 2016; Boguszewska-Mańkowska *et al.* 2020), but unfortunately most breeders still focus on canopy development.

In Vitro Screening of Drought-Tolerant Genotypes Based on Relative Decline (RD) and Susceptibility Index (SSI) Values.

Physiologically, the response of plants to drought stress is characterized as 1) avoidance, i.e., stress does not reach the plant tissue; and 2) tolerance, i.e., stress that enters the plant tissue, but plant tissue can survive (Acquaah 2007). According to Iwama and Yamaguchi (2006), long potato roots are a characteristic of tolerant potato plants. This indicates that potato plants have an avoidance mechanism by having an extensive root system to maintain the turgor potential during drought stress (Acquaah 2007). Potato genotypes differ in root growth and development so there is an opportunity to select the potato genotype which has the root ability to extract water during drought condition (Lahlou & Ledent 2005). However, observing potato roots is very difficult, so according to Gopal & Iwama (2007), the in vitro approach is another alternative method for observing root characteristics. Therefore, it is in agreement with this research that in the screening of tolerant genotypes in vitro, one of the two

Table 5 Response patterns analysis of canopy and root characteristics at five sorbitol levels of seven potato genotypes

Sorbitol (M)	Characteristic					
	PH	CFW	CDW	RL	RFW	RDW
Control	11.77	0.0740	0.00509	11.97	0.0228	0.002280
0.1	7.87	0.0350	0.00329	9.81	0.0149	0.001410
0.2	5.89	0.0267	0.00322	6.63	0.0067	0.000840
0.3	3.28	0.0177	0.00226	2.98	0.0019	0.000024
0.4	1.22	0.0060	0.00084	0.27	0.0001	0.000010
Response Pattern	Lin : 0.000**	Lin : 0.000^{ns}	Lin : 0.000**	Lin : 0.000^{ns}	Lin : 0.000^{ns}	Lin : 0.000**
	Qua : 0.099^{ns}	Qua : 0.0002**	Qua : 0.8227^{ns}	Qua : 0.0001**	Qua :0.0176*	Qua : 0.1810^{ns}

Description: * Significant at α = 0.05, ** Very Significant at α = 0.01, ns: nonsignificant, Lin: Linear, Qua: Quadratic based on orthogonal polynomials. PH: plant height; CFW: canopy fresh-weight; CDW: canopy dry-weight; RL: root length; RFW: root fresh-weight; RDW: root dry-weight.

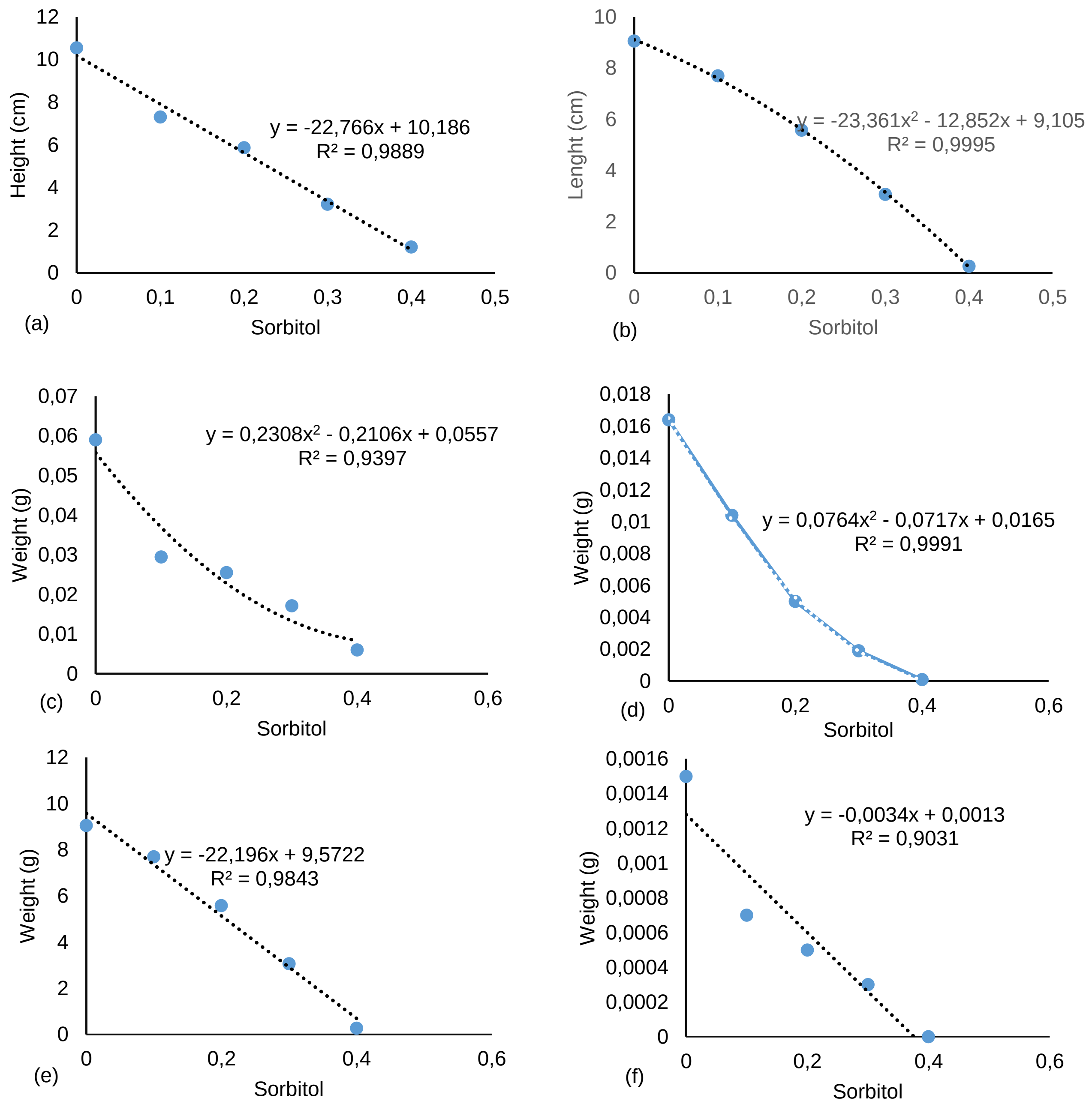

Figure 1 Patterns of sorbitol level effects on the characteristics of (a) plant height = linear; (b) canopy fresh weight = quadratic; (c) canopy dry weight = linear; (d) root length = quadratic; (e) root fresh weight = quadratic; (f) root dry weight = linear.

characteristics that was used as an indicator of selection was the root length. In this study, in vitro screening of tolerant genotypes was performed by calculating the relative decline (RD) and stress susceptibility index (SSI) according to Fischer & Maurer (1978) on both characteristics used as selection indicators, i.e., plant height and root length at 0.2 M sorbitol concentration in which genotypes with the lowest RD and SSI values can be suspected as in vitro tolerant genotypes. Based on the analysis of the relative declines in the two characteristics, the lowest relative declines were PKHT4 and PKHT6, the highest relative decline at 0.2 M concentration for the plant height characteristic was Atlantic genotype, whereas for the root length characteristic, the highest decrease was PKHT9 genotype (Figure 2).

The tolerance level to stress based on SSI according to Kumar *et al.* (2014) can be examined through four categories: highly drought tolerant (SSI < 0.5), drought tolerant (SSI = 0.51–0.75), moderately drought tolerant (SSI = 0.76-1), and drought susceptibility (SSI > 1). The results of the analysis based on stress susceptibility index (SSI) in this study are illustrated in Table 6 that the lowest susceptibility index in the root length was PKHT3 followed by PKHT4 and PKHT6. Based on the plant height, the

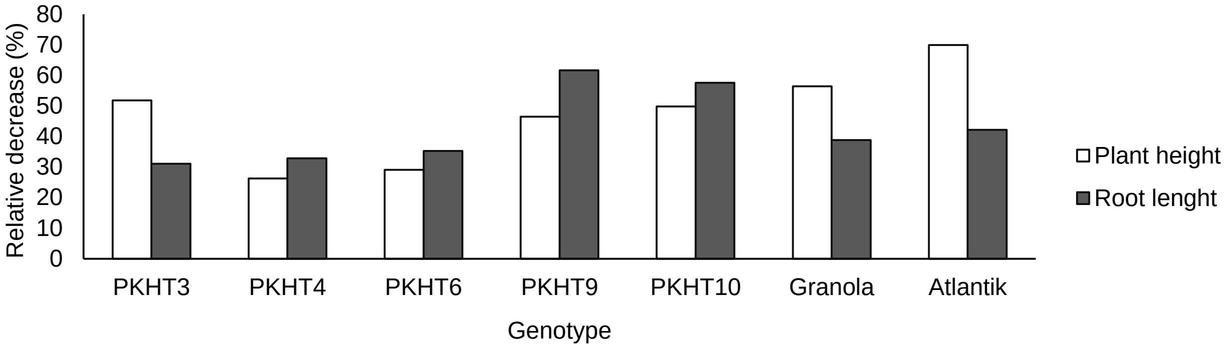

Figure 2 Relative decline (RD) values based on the plant height and root length at 0.2 M sorbitol concentration.

Table 6 Stress susceptibility index (SSI) values of seven potato genotypes in drought stress in vitro

Genotype	SSI Plant height	Category	SSI Root Length	Category
PKHT3	1.037	S	0.697	T
PKHT4	0.526	T	0.736	T
PKHT6	0.528	T	0.791	MT
PKHT9	0.930	MT	1.382	S
PKHT10	0.997	MT	1.491	S
Granola	1.130	S	0.871	MT
Atlantic	1.361	S	0.944	MT

Description: Highly tolerant (ST) = (SSI < 0.5), tolerant (T) = (SSI = 0.51–0.75), moderately tolerance (MT) = (SSI = 0.76–1), drought susceptibility (S) = (SSI > 1).

lowest susceptibility index was PKHT4 followed by PKHT6. However, PKHT3 had a high susceptibility index for plant height characteristic. Therefore, according to the susceptibility index, PKHT4 and PKHT6 genotypes were determined as tolerant genotypes because both had a low susceptibility index value on both selection indicators.

If potato genotypes are different in root growth and development, there is an opportunity to choose potato genotypes that have the root power to obtain water when drought occurs (Levy *et al.* 2013). According to Monneveux *et al.* (2014) short roots are characteristics of susceptible plants. However, for observation of potato roots is very difficult, in which according (Monneveux *et al.* 2014) in vitro approach is another alternative for observing root characteristics. Therefore, in line with this research, in the in vitro screening of tolerant genotypes, one of the two characteristics that will be used as an indicator of selection is the root length.

Based on the RD values of the two characteristics chosen as selection indicators (Figure 2), PKHT4 showed the lowest RD value on both characteristics followed by PKHT6. There were two genotypes, i.e., PKHT10 and PKHT9 which had RD values of plant height characteristic below 50%, but RD values of root length characteristic were more than 50%. PKHT3, Granola, and Atlantic had RD values of root length characteristic were below 50%, but RD values for plant height characteristic for the three genotypes were above 50%. If the genotype has a relative decline below 50 %, there is only one character that is used as a selection indicator then it is not selected as a candidate for the drought-tolerant potato genotype.

Based on the SSI values of the plant height characteristic, PKHT4 and PKHT6 genotypes were tolerant, whereas based on SSI values of the root length characteristic, PKHT3 and PKHT4 were tolerant and PKHT6 was a medium tolerant. However, based on the plant height characteristic, the SSI value of PKHT3 was included to susceptible plant categories. PKHT4 and PKHT6 genotypes were in vitro drought tolerant genotypes. An in vitro assessment of tolerance level with SSI can help for initial screening of drought-tolerant genotypes that must then be verified in vivo. Researches using SSI have been carried out to measure the tolerance level of plants such as Bündig *et al.* (2016) in potato plants in vitro showed that genotypes categorized as tolerant or susceptible will be more tolerant or susceptible compared to in vivo plants.

CONCLUSIONS

In vitro screening media of drought-tolerant potatoes can use MS media added with 0.2 M sorbitol and single nodal as explants. Plant height and root length characteristics can be used as the characteristics that distinguish drought-susceptible and drought-tolerant potatoes. PKHT4 and PKHT6 genotypes were determined as in vitro drought-tolerant genotypes.

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