Plant Cell Rep (2007) 26:693–700
DOI 10.1007/s00299-006-0275-6

BIOTIC AND ABIOTIC STRESS

In vitro screening of potato against water-stress mediated through sorbitol and polyethylene glycol

Jai Gopal · Kazuto Iwama

Received: 30 August 2006 / Revised: 15 November 2006 / Accepted: 17 November 2006 / Published online: 5 January 2007

Abstract With the objective to develop a practical and effective method of screening potato for drought tolerance, shoot and root growth in microtuber-derived plantlets was studied in vitro in three genotypes with known root mass production under field conditions. Different levels of water-stress were induced using five concentrations of either sorbitol or polyethylene glycol (PEG) in MS medium. Water potential of various media ranged from −0.80 MPa to −2.05 MPa. Water-stress in culture adversely affected plantlet growth, and genotypes differed for their responses. Genotype IWA-1 was less affected than IWA-3 and IWA-5. At the same level of water potential, sorbitol had lower adverse effect than PEG; the latter being sticky. Genotype × sorbitol and genotype × PEG interactions were significant. At 0.2 M sorbitol and 0.003 M PEG, IWA-1 had significantly more roots with higher total root length, root volume, as well as root-dry weight than those of IWA-3 and IWA-5, whereas the latter two genotypes were at par for all these characters. This pattern was similar to the reported pattern of these genotypes for root-dry weight under field conditions. It is concluded that in vitro screening of potato under specific and limited water-stress conditions may provide a system for effectively differentiating the genotypes for their expected root mass production under field conditions.

Communicated by P. Kumar

J. Gopal · K. Iwama
Crop Science Laboratory, Research Faculty of Agriculture,
Hokkaido University,
Sapporo, 060-8589 Japan

J. Gopal (✉)
Division of Crop Improvement, Central Potato Research Institute,
Shimla, Himachal Pradesh, 171 001 India
e-mail: jai_gopal54@yahoo.co.in

Keywords Drought tolerance · In vitro rooting · Micropropagation · *Solanum tuberosum*

Abbreviations PEG: Polyethylene glycol · MS: Murashige and Skoog (1962)

Introduction

Water is essential for plant growth. Many physiological processes depend on it. Compared to other species, potato is very sensitive to water-stress because of its shallower root system (Iwama and Yamaguchi 2006). Improvement in root traits is considered to be important for developing drought-tolerant genotypes (Rossouw and Waghmarae 1995; Iwama and Yamaguchi 2006). But, measurement of the root traits in field-grown plants is laborious and time-consuming (Ingram et al. 1994; Erusha et al. 2002; Iwama and Yamaguchi 2006). As a result, little progress has been made in the development of drought-tolerant varieties/cultivars. The normal method of root measurements in field-grown plants needs digging the soil deep (> 1 m), collecting soil cores from different depths, and washing the soil from the roots for recording root characteristics. In vitro approach may be a possible alternative to overcome the problems associated with field evaluation of potato genotypes for root characteristics.

Potato is highly amenable to tissue culture (Espinoza et al. 1986), and micropropagation and microtuberization have become established methods of rapidly multiplying cultivars for seed production as well as for germplasm conservation and exchange (Roca et al. 1979; Ranalli et al. 1994; Gopal et al. 1998, 2002, 2005; Donnelly et al. 2003). The effectiveness of these techniques has also been studied to facilitate selection for tuber characters including yield (Alsadon et al. 1988; Gopal and Minocha 1998; Donnelly et al. 2003),

maturity (Lentini and Earle 1991), and disease resistance (Platt 1992a,b).

Heat and drought tolerance have not been evaluated in vitro in potato (Donnelly et al. 2003). There are, however, some reports on in vitro screening for salinity tolerance (Morpurgo 1991; Zhang and Donnelly 1997; Khrais et al. 1998). The common technique has been to subject single-node stem cuttings, root-tip segments, or microtuberization to salt-stress in vitro. Progressive reduction in plant growth occurs as NaCl levels increase, but there is some inconsistency in the literature concerning the precise level suitable for effective in vitro screening. Thus, to arrive at credible results, evaluation at a range of salinity levels has been suggested (Ashraf 1994; Zhang and Donnelly 1997; Khrais et al. 1998). Osmotic solutions of NaCl, mannitol/sorbitol, and PEG have been used as in vitro stress factors for selecting salt and drought-tolerant genotypes in some other crops also, for example, for seed germination in wheat (Almansouri et al. 2001) and sunflower (Punia and Jain 2002), embryo germination in peach and almond (Ledbetter et al. 1998), sprouting percentage in mulberry (Tewary et al. 2000), nodule development in alfalfa (Djilianov et al. 2003), and growth of nodal cuttings in cassava (Ng and Ekanayake 1997).

Though there is vast literature on the effect of various cultural factors on micropropagation and microtuber production in potato, little information is available on the in vitro root growth. Medium supplemented with NaCl is reported to adversely affect root growth in potato (Zhang and Donnelly 1997). In wheat, drought-tolerant genotypes were found to have better-developed root system under in vitro water-stress mediated through PEG (Ye et al. 2002). In the present study, shoot and root growth in the in vitro plantlets derived by growing microtubers on MS medium (Murashige and Skoog 1962) without and with sorbitol or polyethylene glycol were studied in three potato genotypes with known root mass production under field conditions, with the objective to develop in vitro screening method for drought tolerance.

Materials and methods

The study was conducted at the Crop Science Laboratory of Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan (43°N, 141°E).

Genotypes

Three potato genotypes: IWA-1 (high root-dry weight and very late maturity), IWA-3 (low root-dry weight and late maturity), and IWA-5 (low root-dry weight and medium-early maturity), were used for the study.

Table 1 Media used and their water potentials

Media	Concentration (M)	Water potential (MPa)
Control[a]		− 0.80
with Sorbitol	0.1	− 1.00
	0.2	− 1.35
	0.3	− 1.70
	0.4	− 2.05
with PEG	0.003	− 1.00
	0.006	− 1.10
	0.009	− 1.20
	0.012	− 1.30

[a]MS medium (Murashige and Skoog 1962) with 30 g l^{-1} sucrose and 7 g l^{-1} agar.

Media

Two experiments were conducted, first with sorbitol and second with polyethylene glycol (PEG) as the water-stress inducing agents. In the first experiment, MS (Murashige and Skoog 1962) medium with 30 g l^{-1} sucrose and no hormones was supplemented with five levels of sorbitol (mol. wt. 182.17, Kanto Chemical Co. Inc., Japan), and in the second experiment it was supplemented with five levels of PEG (mol. wt. 8000, SIGMA, USA) (Table 1).

The pH of the media was adjusted to 5.7 ± 1.0, and the media solidified with 7 g l^{-1} agar (Kanto Chemical Co. Inc., Japan). Test tubes (150 mm × 25 mm), each containing 20 ml of the medium and closed with cotton plugs were used for culturing.

Explant and culture conditions

Well-sprouted microtubers (each of 0.3–0.5 g, with a 0.5–1.0 cm long sprout) supplied by Kirin Brewery Co. Ltd. (Japan) were used as explants. These were washed with liquid detergent in running water for 1–2 min, disinfected with 0.1% mercuric chloride for 3 min, rinsed with autoclaved water three times, and cultured aseptically (1 microtuber/test tube).

The cultures were incubated at 24 ± 1°C, under 16 h photoperiod/day providing a photon flux of 80–100 μmol m^{-2} s^{-1} from white fluorescent tubes.

Water potential measurement

Water potential of all media was measured using an isopiestic thermocouple psychrometer (Boyer and Knipling 1965). Measurements were repeated to arrive at a reliable consistent value.

Shoot and root characters

When cultures were 90 days old (without subculturing) and fully grown having stout stems and broad leaves in the control treatment (i.e., MS medium without sorbitol and PEG), data were recorded for various shoot and root characters. At the time of observation, the plantlets, in general, had just started showing signs of senescence (browning of leaf edges and/or shoot tips) and were without any microtuber formation. Plantlets were taken out of the tubes, and number of nodal roots (those originating directly from the basal stem nodes) were counted. Shoot was cut from the roots, and plantlet height was measured as the length of the main stem from base to the tip. Foliage (stems and leaves) was cut into pieces and weighed. Roots were washed to remove any sticking medium and preserved in 70% alcohol, and later measured for root length, root diameter, root volume, and root-dry weight. The former three characters were recorded using an image analysis system (WinRhizo, Regent Instruments Inc., Canada) and root-dry weight was determined after oven-drying the samples to a constant weight at 80°C.

Experimental design and statistical analysis

Both experiments were conducted in a completely randomized two-factor (3 genotypes × 5 levels of osmoticum) factorial design with eight replications. As explant growth was not normal in some replications, the effective number of replications used for data recording was reduced to six. Data were analyzed using the software CPCS1 (Punjab Agricultural University, Ludhiana, India).

Results

Water potentials of the various media used are presented in Table 1. The control semisolid MS medium with 30 g l^{-1} sucrose had a water potential of −0.80 MPa. As expected, the water potential of the media decreased with the addition of sorbitol as well as PEG. Medium with the highest concentration (0.4 M) of sorbitol used in the study had a water potential of −2.05 MPa, whereas it was −1.30 MPa for the medium with the highest concentration (0.012 M) of PEG.

Effect of sorbitol levels on different genotypes

Mean squares due to genotype, sorbitol, and their interaction were significant for plantlet height, foliage fresh weight, number of roots, and roots' length, volume, and dry weight. Performance of the genotypes in media with various levels of sorbitol is presented in Table 2. In the medium with 0.2 M sorbitol, plantlets of IWA-1 were significantly taller (mean 4.5 cm) than those of IWA-3 (1.9 cm) and IWA-5 (1.1 cm), and the difference between the latter two genotypes was not significant. Genotypic differences for plantlet height were not significant in media without sorbitol, and with 0.1 and 0.4 M sorbitol. Plantlet height decreased with increase in sorbitol concentration in all genotypes, but the decrease was more pronounced in IWA-3 and IWA-5 than in IWA-1. Foliage weight of IWA-1 was also higher than those of IWA-3 and IWA-5 in the medium with 0.2 M sorbitol, and the latter two genotypes were at par for this character also. Foliage growth was too little in media with 0.3 and 0.4 M sorbitol in all genotypes (Table 1). Rooting also was adversely affected by increase in sorbitol concentration, and no rooting

Table 2 Effect of different levels of sorbitol on the performance of potato genotypes for various characters

Character	Genotype	Sorbitol concentration (M)				
		0.0	0.1	0.2	0.3	0.4
Plantlet height (cm)	IWA-1	8.8 ab	6.9 c	4.5 d	2.5 e	1.1 fg
	IWA-3	9.6 a	8.1 bc	1.9 ef	1.5 efg	0.78 fg
	IWA-5	8.9 ab	5.4 c	1.1 fg	0.85 fg	0.68 g
Foliage fresh wt. (g/plantlet)	IWA-1	1.1 a	0.87 b	0.41 c	0.04 d	0.03 d
	IWA-3	0.86 b	0.86 b	0.17 d	0.03 d	0.03 d
	IWA-5	0.56 c	0.49 c	0.03 d	0.03 d	0.03 d
Number of roots/plantlet	IWA-1	12.8 a	12.5 a	6.20 d	0.33 e	0.00 e
	IWA-3	12.0 ab	9.8 bc	0.83 e	0.17 e	0.00 e
	IWA-5	11.3 ab	6.0 d	0.67 e	0.17 e	0.00 e
Total root length/plantlet (cm)	IWA-1	564 a	576 a	275 d	24.5 f	0.00 f
	IWA-3	369 c	440 b	160 e	1.00 f	0.00 f
	IWA-5	347 c	294 d	123 e	0.00 f	0.00 f
Root volume/plantlet (cm^3)	IWA-1	0.52 a	0.38 b	0.25 c	0.01 e	0.00 e
	IWA-3	0.42 b	0.37 b	0.15 d	0.00 e	0.00 e
	IWA-5	0.35 b	0.25 c	0.15 d	0.00 e	0.00 e
Roots dry wt. (mg/plantlet)	IWA-1	58.8 a	29.9 de	24.7 d	0.49 f	0.00 f
	IWA-3	47.9 ab	34.1 cd	20.4 e	0.06 f	0.00 f
	IWA-5	43.8 bc	23.5 de	20.4 e	0.12 f	0.00 f

Values followed by same letters (a–g) were not significantly ($P \leq 0.05$) different from each other.

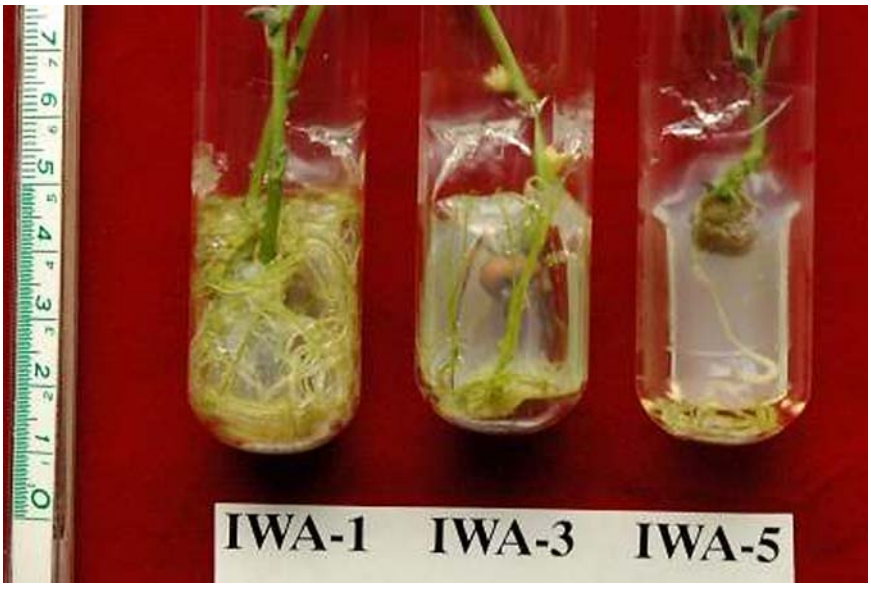

Fig. 1 Root growth in three potato genotypes after 90 days of culture of microtubers on MS medium with 0.2 M sorbitol

was observed in the medium with 0.4 M sorbitol. Genotypes did not differ for root number in the medium without sorbitol, whereas in the medium with 0.2 M sorbitol, IWA-1 had significantly more number of roots (6.2/plantlet) than those of IWA-3 (0.83/plantlet) and IWA-5 (0.67/plantlet), and the latter two genotypes were at par. Like foliage weight, genotypic differences for this trait too were not significant in media with 0.3 and 0.4 M sorbitol.

IWA-1 had significantly longer roots (total root length/plantlet) than those of IWA-3 and IWA-5 in media without sorbitol as well as with 0.2 M sorbitol, and the differences between IWA-3 and IWA-5 were not significant for this character also. Volume and dry weight of the roots/plantlet also were higher in IWA-1 than IWA-3 and IWA-5 in the medium with 0.2 M sorbitol and differences between the latter two genotypes were again not significant (Table 2, Fig. 1). Differences among genotypes for these characters were not significant in media with 0.3 and 0.4 M sorbitol, and for root-dry weight even in the medium with 0.1 M sorbitol. Mean squares for root diameter were significant due to genotypes only. IWA-5 had thinner roots (mean diameter 0.28 mm) than those of IWA-1 (0.31 mm) and IWA-3 (0.32 mm).

Effect of PEG levels on different genotypes

Mean squares due to genotype, PEG, and their interaction were significant for plantlet height, foliage fresh weight, and roots' length, diameter, volume, as well as dry weight. Effect of PEG levels on genotype performance for these characters is presented in Table 3. In the medium with 0.006 M PEG, plantlets of IWA-1 were significantly taller (9.2 cm) than those of IWA-3 (7.5 cm) and IWA-5 (6.5 cm), whereas the latter two genotypes did not differ significantly. Plantlet height decreased with increase in the concentration of PEG, and the decrease was more pronounced in IWA-3 and IWA-5 than in IWA-1. In media with 0.009 and 0.012 M PEG, plantlets were significantly shorter than those in lower concentrations of PEG, and the genotypic differences were not significant in the medium with 0.012 M PEG. Like plantlet height, foliage weight also decreased drastically in media with >0.006 M PEG and the genotypic differences were not significant. In media with up to 0.006 M PEG, IWA-1 had significantly more foliage weight than IWA-3 and IWA-5, whereas the latter two genotypes did not differ significantly.

All root characters also were adversely affected by increase in PEG concentration. In the medium with 0.003 M PEG, IWA-1 had higher root length, root diameter, root volume, as well as root-dry weight than those of IWA-3 and IWA-5, and the latter two genotypes were at par with each other. In medium without PEG, genotypic differences were not significant for all these characters. In other media too, genotypic differences for these characters were not significant, except for root-dry weight which was higher in IWA-1 than IWA-3 and IWA-5 in media with 0.006 and 0.009 M PEG, and root diameter which was higher in IWA-3 and IWA-5 than IWA-1 in the medium with 0.006 M PEG, whereas the reverse was true in the medium with 0.009 M PEG.

Genotype $\times$ PEG interaction was not significant for number of roots per plantlet. Mean number of roots over media were more in IWA-1 (8.6/plantlet) than IWA-3 (4.1) and IWA-5 (3.7), and the latter two genotypes were at par with each other. Mean number of roots per plantlet over genotypes was 10.5 in the medium without PEG and this decreased to 1.2 in the medium with 0.012 M PEG. Only IWA-1 could produce some roots (3.5/plantlet) in the medium with 0.012 M PEG.

Table 3 Effect of different levels of polyethylene glycol on the performance of potato genotypes for various characters

Character	Genotype	PEG concentration (M)				
		0.000	0.003	0.006	0.009	0.012
Plantlet height (cm)	IWA-1	10.5abc	9.3 c	9.2 c	5.4 ef	4.3 fg
	IWA-3	11.6 a	9.8 bc	7.5 d	4.1 fg	3.6 g
	IWA-5	8.0 cd	6.1 e	6.5 de	3.9 g	3.0 g
Foliage fresh wt. (g/plantlet)	IWA-1	1.20 a	0.77 b	0.51bcd	0.20 ef	0.09 f
	IWA-3	0.64 bc	0.30 def	0.11 f	0.03 f	0.03 f
	IWA-5	0.81 b	0.42 cde	0.27 def	0.01 f	0.04 f
Total root length/plantlet (cm)	IWA-1	550 a	522 a	356 b	114 ef	69 fg
	IWA-3	470 a	270 bc	270 bc	44 fg	0.0 g
	IWA-5	485 a	253 cd	263 bcd	49 fg	0.0 g
Root diameter (mm)	IWA-1	0.28 h	0.37 bcd	0.39 bc	0.45 a	0.36 bcde
	IWA-3	0.31 efgh	0.32 efgh	0.45 a	0.33 defgh	0.35 cdef
	IWA-5	0.30 fgh	0.29 gh	0.41 ab	0.35 cdefg	0.33 defgh
Root volume/plantlet (cm^3)	IWA-1	0.42 b	0.59 a	0.42 b	0.18 def	0.07 fg
	IWA-3	0.36 bc	0.32 bc	0.44 b	0.12 efg	0.00 g
	IWA-5	0.34 bc	0.25 cde	0.31 bcd	0.08 fg	0.00 g
Roots dry wt. (mg/plantlet)	IWA-1	49.5 ab	47.3 abc	49.5 ab	23.4 def	8.20 fgh
	IWA-3	33.8 bcde	31.9 cde	30.7 de	6.0 gh	0.00 h
	IWA-5	34.6 bcde	31.2 cde	31.7 cde	5.4 gh	0.00 h

Values followed by same letters (a–g) were not significantly ($P \leq 0.05$) different from each other.

Discussion

The addition of sorbitol or PEG to the MS medium decreased the water potential of the media inducing water-stress that adversely affected both shoot and root growth of the plantlets. Water-stress under field conditions is also known to adversely affect plant growth including stem height, foliage weight, root number, and root-dry weight along with a corresponding decline in tuber yield (Munns and Pearson 1974; Lynch and Tai 1989; Deblonde and Ledent 2001; Tourneux et al. 2003; Lahlou and Ledent 2005). So, the general effect of water-stress on in vitro plant growth as observed in the present study was similar to that observed under the field conditions. These similarities in the effects of water-stress under in vitro and in vivo conditions suggest that the in vitro system can be used as an alternative to field evaluations for studying the general effect of water-stress on plant growth and development. The observed differences between the two experiments for the performance of various characters on the same control MS medium (Tables 2 and 3) may be due to the fact that physiologically older microtubers were used in the second experiment as it was initiated 2 months later than the first one.

The three genotypes used in the present study, had been developed at the Hokkaido University, Japan and previously evaluated for root-dry weight under field conditions. Based on 3-year data, IWA-1 was found to have significantly higher root-dry weight (4.7 g/plant) than that of (2.6 g/plant) IWA-3 (Iwama et al. 1999). The difference between IWA-5 (2.0 g/plant) and IWA-3 for root-dry weight was not significant, but IWA-5 matures earlier than IWA-3 (unpublished). IWA-3 is late maturing and IWA-1 is very late maturing (Iwama et al. 1999). Genotypes with longer maturity period, in general, have high root-dry weight under field conditions, but in genotypes maturing early, root growth stops earlier (Iwama 1998). The genotypes used in the present study represented the different possible combinations of root-dry weight and foliage maturity. A genotype with high or medium root-dry weight and early maturity perhaps is too difficult to find (Iwama et al. 1981). The results of the present study show that root-dry weight of the three genotypes under field conditions was very well reflected by their root growth in vitro on MS medium supplemented with 0.2 M Sorbitol or 0.003 M PEG. In these media, IWA-1 had more number of roots with higher total root length, root volume, as well as root-dry weight than those of IWA-3 and IWA-5, and the latter two genotypes did not differ from each other significantly for any of these characters (Tables 2 and 3). Similar pattern of differences were also observed for plantlet height and foliage fresh weight.

IWA-1 had higher root-dry weight than IWA-3 and IWA-5 also in the media supplemented with some other concentrations of sorbitol or PEG. However, in the medium without sorbitol and PEG, or having more than 0.2 M of sorbitol or more than 0.003 M of PEG, genotypic differences for one or more of root as well as shoot characters were not significant. Under extreme water-stress conditions as induced by 0.4 M sorbtiol and 0.012 M PEG, all genotypes had little shoot as well as root growth (Tables 2 and 3) and genotypic differences were not significant for any character. Thus, the proposed in vitro system for screening potato against water-stress has the limitation that water-stress induced to only a limited level can be used for differentiating the genotypes. These results have similarity to findings with single-node cuttings of potato screened for salinity tolerance where use of

a relatively high level of NaCl failed to quantify differences among cultivars in vitro (Morpurgo 1991). Water-stress beyond the limit of tolerance by a plant will naturally prove fatal, as is also observed under field conditions. Differential growth response observed at different water-stress levels in vitro, is consistent with similar observations found in vivo (Iwama et al. 1999), and suggests the involvement of different genes at different levels of stress (Tal 1994). This and significant genotype × water-stress (sorbitol/PEG) interaction for a majority of the characters as observed in the present study suggest that ranking of a genotype at a particular level of water-stress would not be predictive of its water-stress tolerance at other levels applied in culture or in the field. Relative vigor is an important component of tolerance to abiotc stress (Munns 1993). Higher foliage weight of IWA-1 in medium without sorbitol or PEG suggests that relative plantlet growth may be sufficient to select for both vigor and root geometry such as root length—both of which are useful under water-stress conditions. However, keeping in view the consistent pattern of genotypic differences for all characters, MS medium with 0.2 M sorbitol or 0.003 M PEG can be considered as the best for reliably differentiating the genotypes for their expected root-dry weight under field conditions. This can be based on the in vitro root-dry weight or related characters, i.e., root number, total root length, or root volume. Among these, root-dry weight, which reflects the overall root growth, perhaps is also the easiest to measure reliably. Since genotypic difference for root diameter did not follow the pattern observed for other root characters in various media tested, this parameter is not useful for differentiating the genotypes for their overall root growth.

The results also showed that the observed adverse effect of water-stress on shoot and root growth, was in general lower on IWA-1 than IWA-3 and IWA-5 over a range of stress levels, thus indicating higher drought-tolerance ability of IWA-1. Under water-stress field conditions too, IWA-1 had higher root-dry weight than IWA-3 (Iwama et al. 1999). However, IWA-5 was not included in the study. Root fresh weight in vitro (Morpurgo 1991) as well as in vivo (Iwama et al. 1982) is reported to be positively correlated with tuber yield in vivo. Under field conditions, larger root-dry weight delays leaf senescence and thus prolongs tuber bulking (Iwama et al. 1982). So, the observed relationship of growth under mild water-stress (induced by 0.2 M sorbitol or 0.003 M PEG) in culture in three genotypes with their relative growth under both nonstress and water-stress field conditions is due to the inherent higher drought-tolerance ability of IWA-1 compared with IWA-3 and IWA-5.

The nature of the water-stress inducing agent is also important for root growth. It is desirable to use a compound that does not interact with plants in any way other than lowering the water potential of the medium. Thus, slowly penetrating osmotica such as mannitol or sorbitol (Hohl and Schopfer 1991) or inorganic salts (Termaat and Munns 1986) are not ideal, especially for experiments extending beyond a few hours. Polymers of PEG have been used for many years, principally because PEG molecules with molecular weight ≥ 6000 cannot penetrate the cell wall pores (Carpita et al. 1979). Because PEG does not enter the apoplast, water is withdrawn not only from the cell but also from the cell wall. Therefore, PEG solutions mimic dry soil more closely than solutions of low molecular weights osmotica, which infiltrate the cell wall with solute (Verslues et al. 1998). However, PEG has its own drawbacks. Some studies have indicated that PEG could contain toxic contaminants that inhibit plant growth (Plaut and Federman 1985). Another potential disadvantage is that the high viscosity of PEG solutions limits the movement of O_2, thereby increasing the likelihood of root O_2 deficiency. In the present study, the water potential of the medium with 0.2 M sorbitol was -1.35 MPa, and though it was similar to that caused by 0.012 M PEG (Table 1), the latter medium had higher adverse effect on plant growth, and genotypes did not differ for various characters in this medium. PEG being very viscous, the media supplemented with this were found to very sticky and hard, and also difficult to wash off from the roots. Thus, it is not only the water potential imposed by the different stress inducing agent, but also the texture of the medium that affects the plant growth, especially the root development. Keeping in view the ease with which the medium can be washed off the roots, and lower adverse effect on plant growth at the same level of water potential, sorbitol would be a better choice for in vitro induction of water stress.

In conclusion, in vitro propagation system using MS medium with 0.2 M sorbitol and microtubers as explant (Fig. 1), may have potential to differentiate potato genotypes for their root-dry weight production under field conditions. The effectiveness of this system, however, should be further tested on a greater number of genotypes with known performance for root characteristics related to drought-tolerance under field conditions. One issue in comparing effectiveness of screening methods in relation to field rankings for drought-tolerance is to determine the reliabilty of the rankings. In such comparisons, data from field evaluations must be based on well-conducted trials repeated over years. Unfortunately, such evaluations for root characters are available for only a few genotypes. Nevertheless, the results of the present study clearly showed that screening of genotypes under cultural conditions that simulate the in vivo conditions might provide a high efficacy in vitro screening method for abiotic stresses like drought tolerance.

Acknowledgments Dr. Jai Gopal is thankful to the Japan Society for Promotion of Science (JSPS) for the award of Invitation Fellowship, and to the Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan for inviting him as Visiting Professor. We are thankful to Dr.

Y. Jitsuyama for help in arranging the facilities, and to Mr. Suita, Ms. Dunia, and Mrs. Katayama for their kind help in undertaking this study. We are also thankful to Mr. Noboru Ohnishi of Kirin Brewery Co. Ltd., Japan for supplying the microtubers.

References

Alasdon AA, Knutson KW, Wilkinson JC (1988) Relationship between microtuber and minitubers production and yield characteristics of six potato cultivars. Am Potato J 65:468 (abst)

Almansouri M, Kinet JM, Lutts S (2001) Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum*) Desf . Plant Soil 231:243–254

Ashraf M (1994) Breeding for salinity tolerance in plants. CRC Crit Rev Plant Sci 13:17–42

Boyer JS, Knipling EB (1965). Isopiestic technique for measuring leaf water potential with a thermocouple psychrometer. Proc Natl Acad Sci USA 54:1044–1051

Carpita N, Sabularse D, Montezinos D, Delmer DP (1979) Determination of the pore size of cell walls of living plant cells. Science 205:1144–1147

Deblonde PMK, Ledent JF (2001) Effects of moderate drought conditions on green leaf number, stem height, leaf length and tuber yield of potato cultivars. Eur J Agron 14:31–41

Djilianov D, Prinsen E, Oden S, Onckelen H van, Muller J (2003) Nodulation under salt stress of alfalfa lines obtained after in vitro selection for osmotic tolerance. Plant Sci 165:887–894

Donnelly DJ, Coleman WK, Coleman SE (2003) Potato microtuber production and performance: a review. Am J Potato Res 80:103–115

Erusha KS, Shearman RC, Roirdan TP, Wit LA (2002) Kentucky bluegrass cultivar root and top growth responses when grown in hydroponics. Crop Sci. 42:848–852

Espinoza NO, Estrada R, Silva-Rodriguez D, Tovar P, Lizarraga R, Dodds JH (1986) The potato: a model crop plant for tissue culture. Outlook Agric 15:21–26

Gopal J, Minocha JL (1998) Effectiveness of in vitro selection for agronomic characters in potato. Euphytica 103:67–74

Gopal J, Minocha JL, Dhaliwal HS (1998) Microtuberization in potato (*Solanum tuberosum* L.). Plant Cell Rep 17:794–798

Gopal J, Chamail A, Sarkar D (2002) Slow-growth in vitro conservation of potato germplasm at normal propagation temperature. Potato Res 45:203–213

Gopal, J., Chamail A, Sarkar D (2005) Use of microtubers for slow-growth in-vitro conservation of potato germplasm. Plant Genet Resour Newsl 141:56–60

Hohl M, Schopfer P (1991) Water relations of growing maize coleoptiles. Comparison between mannitol and polyethylene glycol 6000 as external osmotica for adjusting turgor pressure. Plant Physiol 95:716–722

Ingram KT, Bueno FD, Namuco OS, Yambao EB, Beyrouty CA (1994) Rice root traits for drought resistance and their genetic variation. In: Kirk GJD (ed) Rice roots: nutrient and water use. IRRI, Manila, Philippines, pp 67–77

Iwama K (1998) Development of nodal and lateral roots in potato under field conditions. J Fac Agri Hokkaido Univ 68:33–44

Iwama K, Yamaguchi J (2006) Abiotic stresses. In: Gopal J, Khurana SM Paul (eds) Handbook of potato production, improvement and postharvest management. Food Product Press, New York, pp 231–278

Iwama K, Isoda A, Gotoh K, Nishibe S (1981) Relations between root system and tuber yield in the hybrid population of the potato plants. Jpn J Crop Sci 50:233–238

Iwama K, Nakaseko K, Gotoh K, Nishibe Y (1982) Studies on the root system in potato plants. In: Hooker WJ (ed) Research for potato in the year 2000. International Potato Center, Lima, Peru, pp 102–104

Iwama K, Hasegawa T, Nakaseko K (1999) New potato lines with high productivity and drought tolerance. In: Horie H, Geng S, Amano T, Inamura T, Shiraiwa T (eds) Proceedings of the international symposium on world food security and crop production technologies for tomorrow, Kyoto, Japan, pp 189–193

Khrais T, Leclerc Y, Donnelly DJ (1998) Relative salinity tolerance of potato cultivars assessed by in vitro screening. Am J Potato Res 75:207–210

Lahlou O, Ledent JF (2005) Root mass and depth, stolons and roots formed on stolons in four cultivars of potato under water stress. Eur J Agron 22:159–173

Ledbetter CA, Palmquist DE, Peterson SJ (1998) Germination and net in vitro growth of peach, almond and peach-almond hybrid embryos in response to mannitol inclusion in the nutrient medium. Euphytica 103:243–250

Lentini Z, Earle ED (1991) In vitro tuberization of potato clones from different maturity groups. Plant Cell Rep 9:691–695

Lynch DR, Tai GCC (1989) Yield and yield component response of eight potato genotypes to water stress. Crop Sci 29:1207–1211

Morpurgo R (1991) Correlation between potato clones grown in vivo and in vitro under sodium chloride stress conditions. Plant Breed 107:80–82

Munns R (1993) Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant Cell Environ 16:15–24

Munns R, Pearson CJ (1974) Effects of water deficit on translocation of carbohydrate in *Solanum tuberosum*. Aus J Plant Physiol 1:529–537

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant 15:473–497

Ng SYV, Ekanayake IJ (1997) Screening cassava (*Manihot esculenta* Crantz) for polyethylene glycol mediated osmotic stress in vitro. J Root Crops 23:15–18

Platt HW (Bud) (1992a) Cultivar response to fusarium storage rot as affected by two methods of seed origin propagation; clonal selections and in vitro culture. Am J Potato Res 69:179–186

Platt HW (Bud) (1992b) Potato cultivar response to late blight as affected by clonal selections and in vitro culture. Am J Potato Res 69:187–193

Plaut Z, Federman E (1985) A simple procedure to overcome polyethylene glycol toxicity on whole plants. Plant Physiol 79:559–561

Punia MS, Jain A (2002) In vitro selection for drought tolerance in sunflower (*Helianthus annus* L.). Nat J Plant Improv 4:27–30

Ranalli P, Ruaro BG, Delre P, Dicandilo M, Mandilino G (1994) Microtuber and minitubers production and field performance compared with normal tubers. Potato Res 37:383–391

Roca WM, Bryan JE, Roca MR (1979) Tissue culture for international transfer of potato genetic resources. Am Potato J 55:1–10

Rossouw FT, Waghmarae J (1995) The effect of drought on growth and yield of two South African potato cultivars. S Afr J Sci 91:149–150

Tal M (1994) In vitro selection for salt tolerance in crop plants: theoretical and practical considerations. In Vitro Cell Dev Biol 30:175–180

Termaat A, Munns R (1986) Use of concentrated macronutrient solutions to separate osmotic from NaCl-specific effects on plant growth. Aust J Plant Physiol 13:509–522

Tewary PK, Sharma A, Raghunath MK, Sarkar A (2000) In vitro response of promising mulberry (*Morus* sp.) genotypes for tolerance to salt and osmotic stresses. Plant Growth Regul 30:17–21

Tourneux C, Devaux A, Camacho MR, Mamani P, Ledent JF (2003) Effects of water shortage on six potato genotypes in the highlands of Bolivia (I): morphological parameters, growth and yield. Agronomie 23:169–179

Verslues PE, Ober ES, Sharp RE (1998) Root growth and oxygen relations at low water potentials. Impact of oxygen availability in polyethylene glycol solutions. Plant Physiol 116:1403–1412

Ye X, Du L, Xu H, Xin Z (2002) Root characters of several wheat genotypes. J Triticeae Crops 22:43–46

Zhang Y, Donnelly DJ (1997) In vitro bioassays for salinity tolerance screening of potato. Potato Res 40:285–295