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USE OF POLYETHYLENE GLYCOL (PEG) 8000 FOR IN VITRO SCREENING OF POTATO GENOTYPES FOR DROUGHT TOLERANCE: I. ROOT AND SHOOT GROWTH[1)]

PENGGUNAAN POLYETHYLENE GLYCOL (PEG) 8000 DALAM SELEKSI KETAHANAN TANAMAN KENTANG TERHADAP CEKAMAN KEKERINGAN SECARA *IN VITRO*: I. PERTUMBUHAN AKAR DAN PUCUK[2)]

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ABSTRACT

The overall goal of this study was to evaluate whether growth reduction of potato genotypes, expressed in root length density reduction (RLDR), root dry weight reduction (RDWR), shoot dry weight reduction (SDWR), root-to-shoot ratio reduction (RSR) could be used to select potato genotypes for drought tolerance. Twelve potato genotypes grown in vitro were exposed to 0% and 8% PEG8000, their growth reductions at 8% PEG8000 were calculated, and then were ranked from the least and the most reduced in growth.

The results showed that Kennebec, known to be drought tolerant in previous studies, showed the least growth reduction as seen in the RLDR (12.4% and 20.9%), RDWR (20.4% and 38.1%), SDWR (11.7% and 47.8%, and RSR (-120.1% and –18.2%). Furthermore, the linear correlations of RLDR (r^2 = 0.89*) and RDWR (r^2 = 0.78*) results over runs of the experiment were significant, suggesting that the results were consistent. RLDR and RDWR show promise for selecting potato genotypes grown in vitro for drought tolerance. The linear relationship between RLDR and RDWR was also significant (r^2 = 0.86*), suggesting that either one was good for screening.

Key words: PEG, in vitro screening, water stress, potato, root-to-shoot ratios

INTISARI

Penelitian ini bertujuan untuk mengevaluasi apakah penurunan pertumbuhan genotipe-genotipe kentang, yang dilihat dari penurunan densitas panjang akar, penurunan berat kering akar, penurunan berat kering tajuk, penurunan rasio akar-tajuk, dapat digunakan untuk seleksi genotipe

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kentang yang toleran terhadap kekeringan. Dua belas genotipe kentang ditanam secara in vitro dan diperlakukan dengan 0% dan 8% PEG8000. penurunan pertumbuhan pada perlakuan 8% PEG diukur dan disusun dari yang paling sedikit hingga paling banyak mengalami penurunan pertumbuhan.

Hasil penelitian menunjukkan bahwa Kennebec, yang telah diketahui toleran terhadap kekeringan pada penelitian sebelumnya, menunjukkan penurunan pertumbuhan yang paling sedikit, seperti yang terlihat pada RLDR (12.4% dan 20.9%), RDWR (20.4% dan 38.1%), SDWR (11.7% dan 47.8%, dan RSR (-120.1% dan –18.2%). Lebih lanjut, korelasi linear RLDR (r^2 = 0.89) and RDWR (r^2 = 0.78*) yang diperoleh berbeda nyata. Hal ini menunjukkan bahwa hasil yang diperoleh konsisten. Dengan demikian RLDR dan RWDR menjanjikan untuk seleksi in vitro genotype kentang toleran terhadap kekeringan. Korelasi linear antara RLDR dan RWDR juga berbeda nyata (r^2 = 0.86*) sehingga salah satu dari kedua parameter tersebut cukup untuk seleksi.*

Kata kunci: PEG,seleksi in vitro, cekaman kekeringan, kentang, rasio akar-tajuk.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is well known to be very sensitive to drought stress (Ekanayake and de Jong, 1992). Part of the reason is due to its poor soil water extraction (Weisz *et al.*, 1994) as a result of the shallow and ineffective rooting system. Most of the roots are confined at the upper 30 cm soil layer (Opena and Porter, 1999). On the other hand, drought-adapted plants are characterized by deep and vigorous root systems. These rooting systems are associated with extensive rooting depth, high root length density, and low resistance to water flow within the root (Monneveux and Belhassen, 1996).

Plants experience drought by excessive transpiration and/or by a limitation of water supply (Frensch, 1997). Although drought stress reduces plant water potentials (y_s), it affects root and leaf growth differently (Frensch, 1997). Many studies have shown that root growth is more resistant to water deficit than shoot growth (Frensch, 1997; Hsiao and Xu, 2000). Furthermore, drought stress increases both root-to-shoot ratio and root-length-to-root weight ratio (Jefferies, 1993).

It has been documented that drought stress reduces plant growth (Weisz *et al.*, 1994), marketable yield, tuber number per stem, and average tuber yield (Lynch and Tai, 1989), carbohydrate accumulation and partitioning (Ekanayake and de Jong, 1992), the yielding capacity of potato crops, and

subsequent performance of the seed tubers (Karafyllidis, 1996). Moreover, drought stress has been reported to reduce gas exchange, decrease the concentration of phosphorylated intermediates, like 3-phosphoglycerate acid (3PGA) and inhibit starch synthesis (Geigenberger *et al.*, 1999). Other studies have shown that drought stress increases the incidence of internal tuber defects (Miller and Martin, 1985), increases the percentage of sugar-end tubers (Kincaid *et al.*, 1993), and increases total glycoalkaloid content (Papathanasiou *et al.*, 1999).

So significant is the effect of drought stress on potato growth and yield that the need for genotypes adapted to drought has become urgent (Maldonado *et al.*, 1998; Rajashekar *et al.*, 1995). In fact, there have been major efforts to develop drought-tolerant cultivars. Through intensive breeding programs, researchers have successfully released some potato cultivars that are drought tolerant. However, conventional breeding techniques are considered to be painstaking and time consuming. It may take 10 to 15 growth cycles from crossing of parental lines to the final release of new cultivars (Caligari, 1992). Therefore, it is of importance to develop rapid screening techniques to shorten the time spent in breeding.

Some rapid methods for screening drought-tolerance traits in potato have been established (Bansal *et al.*, 1991; Demagante *et al.*, 1995). Canopy temperature and chlorophyll *a* fluorescence have been reported as potential tools for drought screening of potato germplasm (Jefferies, 1992; Ranalli *et al.*, 1997; Stark *et al.*, 1991). Demagante *et al* (1995) employed apical cuttings for screening drought tolerance in raised beds. Bansal *et al.* (1991) established a new screening method by using the growth reduction of leaf discs floated over different concentrations of polyethylene glycol (PEG) 6000 (now PEG8000, Sigma Aldrich, 2001).

In vitro bioassays have been employed to screen potato genotypes for salinity tolerance (Zhang and Donnelly, 1997), to screen *Prunus* tolerance to osmotic stress (Rajashekar *et al.*, 1995), and to select drought-tolerant rice (Biswas *et al.*, 2002). Even though *in vitro* techniques can potentially be used to screen potato genotypes for drought tolerance, no such research has been reported

The purposes of this experiment were to study whether genotypes, PEG8000 concentration, and their interaction affected the growth of potato crops, and to evaluate whether root and shoot growth of potato genotypes grown at low water potentials (y_w) can be used as tools for screening potato for drought tolerance.

MATERIAL AND METHODS

Plant materials used in this experiment were obtained from the International Potato Center, CIP, (Chagllina-INIA, E86.011, Reiche, C89.315, Tacna, and Unica) and from Dr. Feridoon Mehdizadegan, the Maine Seed

Potato Board (Andover, Superior, Shepody, Kennebec, Katahdin, and Russet Burbank).

The potato genotypes were exposed to different artificially imposed water potentials by adding 0 or 8% polyethylene glycol (PEG) 8000 to the culture media. Two single-node cuttings, 1-cm long with one leaf and one axillary bud, taken from the medial part of 3-week-old micro-propagated plantlets, were cultured in 25mm x 125mm Pyrex glass test tubes, containing 10 ml of potato micro-propagation culture media at designated PEG8000 concentrations. The plant materials were previously grown in test tubes containing 10 ml solid media (Zhang and Donnelly, 1997) and sub-cultured every 8 weeks since they arrived at the University of Maine from either CIP in February 2000 or the Maine Seed Potato Board in fall 1999.

The culture media were prepared by following Zhang and Donnelly (1997) in which a modified MS (Murashige and Skoog, 1962) basal salt solution was supplemented with inositol (100 mg l^{-1}), pyridoxine·HCl (0.5 mg l^{-1}), thiamine·HCl (1.0 mg l^{-1}), niacin (0.5 mg l^{-1}), Ca-pantothenate (2.0 mg l^{-1}), glycine (2.0 mg l^{-1}), 3% sucrose and 0.6% agar. The medium was adjusted to pH 5.7 prior to autoclaving at 121 °C for 20 minutes.

The cultures were incubated at 25 °C with 16/8 day/night at 40 µmol m^{-2} s^{-1} photon flux density of cool-white fluorescent light. After six weeks of incubation, the plantlets were harvested for total root length (RL), shoot length (SL), root dry weight (RDW), and shoot dry weight (SDW),

The experiment was conducted twice and arranged in a randomized complete block design (2 factors) with five replications per treatment. The first experiment was carried out from May to July 2001 and the second set from September to November 2002. Data analyses were done using Proc. GLM (SAS Institute, Cary, NC) for analysis of variance, followed by mean separation with Duncan's Multiple Range Test, in addition to linear correlation analysis done with Microsoft Excel (with *r critical* of *0.58;* α *= 0.05; n = 12*).

RESULTS AND DISCUSION

Effect of Genotypes.

Root length density (RLD), root dry weight (RDW), shoot dry weight (SDW), and root-to-shoot ratio (RS) were all significantly affected by potato genotypes (Table 1) in both years. Five genotypes (E86.011, Andover, Chaglina-INIA, Reichie, and Unica) consistently belonged to the group with high RLD values over the years. There was no consistency regarding which genotype showed the lowest RLD values over years. For example, in 2001 the lowest RLD was found in Shepody, while in 2002 it was found in Kennebec. So far, there has been no published information on the effect of water stress on RLD of potato crops grown *in vitro* to which the author might

compare the results of current study. From their field studies, Vos and Greenwold (1986) reported RLD values of 1 and 2 cm cm^{-3} in the uppermost soil layer, and Lesczynski and Tanner (1976) found the typical RLD values of 2 to 6 cm cm^{-3} in the uppermost soil layer. In a more recent field study, Opena and Porter (1999) reported that the RLD values of Superior in the 0-15 soil layer of control treatments were 1.23 and 1.96 cm cm^{-3} in 1993 and 1994. In this experiment, the RLD values of Superior were 3.89 and 1.95 cm cm^{-3} for 2001 and 2002, respectively (Table 1). However, one should keep in mind that the growing environment of a test tube with culture media is very different from that of a soil.

The highest values of root dry weight (RDW) were found in Reichie, Unica, and Andover, and the RLD of these varieties were also among the highest (Table 1). Furthermore, Katahdin whose RLD was among the lowest in both years, showed the lowest RDW value, followed by Shepody. These results were consistent with a previous study of Opena and Porter (1999), in which increasing RLD due to soil amendment also increased RDW. However, this kind of consistency was not found in the other genotypes used in this experiment.

Shoot dry weight (SDW) was significantly affected by potato genotypes for both years. It ranged from 7.36 to 76.65 mg in 2001 and from 1.69 to 21.21 mg in 2002, with the highest values was found in Reichie, Superior, Tacna, and Andover in 2001 and Reichie in 2002 (Table 1). Considering the main function of the root in *in vitro* culture, which absorbs water, nutrients, and sugars (Kyte, 1999), it is likely that the performance of rooting systems directly contribute to the growth of the plants, as seen in Reichie, Andover, Katahdin, and Russet Burbank.

The root-to-shoot ratio (RS) values of this experiment ranged from 0.09 to 0.86 in 2001 and from 0.13 to 0.70 in year 2002, which were much higher than those reported by Opena and Porter (1999) in their field experiment (0.04 and 0.08 for two consecutive years). The difference in the growing environment might contribute to these differences. In our experiment, potato plants were grown in very rich and soft growth media (Zhang and Donnelly, 1994). In the field experiment, potato root growth may be inhibited by poor soil texture, lack of nutrients, and the presence of physical impedance (Opena and Porter, 1999; Vos and Greenwold, 1986). Furthermore, under favorable field conditions, potato crops would allocate more dry weight to the shoot than to the root (and hence low RS values) to maximize the light harvesting capacity of the crops, required for high tuber yield. This was unlikely the case for potato plants grown *in vitro* that did not need to produce their own sugars (Kyte, 1987).

In general, the genotypes performed better in 2001 than in 2002 as shown by the RLD, RDW, and SDW. The reason was not clear. It might have

been due to the differences in the physiological age and/or the number of subcultures of plant materials used to start the experiment. Plant materials used in 2002 were at least a year older and had three more subcultures than those used in 2001. A previous study showed that an increase in the number of subcultures of potato plantlets and/or the physiological age of mother tubers significantly reduced plantlet growth (Villafranca *et al.*, 1998).

Effect of PEG8000.

As expected, PEG treatments significantly reduced plant growth (Table 1) for both years, except for RS in 2001. When applied to growth media, PEG8000 acted to hold water mimicking the effect of water stress (Bansal *et al.*, 1991; Michel and Kaufmann, 1973), which might result in the reduction in nutrient and water absorption by the roots. The results of this experiment confirmed previous studies using PEG for introducing water stress (Steuter *et al.*, 1981; Bansal *et al.*, 1991).

It was expected that water stress would increase the RS values of the crops. However, the results of this experiment indicated that water stress (8% PEG8000) did not significantly increase RS value, even though there was a slight increase in RS for 2001 (Table 1). In 2002, water stress (8% PEG8000) significantly reduced RS. Perhaps it was due to the increase in the number of subcultures of the plants used to start the experiment as mentioned above (Villafranca *et al,* 1998).

Interaction between Genotype and PEG8000.

The effects of PEG8000 on root length density (RLD), root dry weight (RDW), shoot length (SL), shoot dry weight (SDW), and root-to-shoot ratio (RS), were dependent on the potato genotypes (Table 1). The ultimate goal of this experiment was to evaluate whether root and shoot growth of potato crops grown *in vitro* could be used as tools for screening potato genotypes for drought-stress tolerance. Therefore, the authors focused the discussion on the growth reduction of the genotypes at 8% PEG8000, instead of the actual growth at that level of PEG. The growth of potato crops at 8% PEG8000 was compared to the control (0% PEG8000) treatments, then the genotypes were ranked from the least (1) to the most (12) reduced to determine their relative degree of drought tolerance (Bansal *et al.*, 1991). It was expected that the more tolerant genotypes would show less growth reduction than the more sensitive ones, as the degree of reduction in growth was considered as an index of drought stress (Demagante *et al.*, 1995).

Water stress (8% PEG8000) caused a significant reduction in root length density (RLDR), in which Kennebec was the lowest in both years (Table 2.). The RLDR values for Kennebec in this experiment were

comparable to that reported by Bansal *et al.* (1991) in their leaf disc experiment, in which Kennebec showed growth reduction of 27% when exposed to –0.4 MPa. In their experiment, Bansal *et al* (1991) also found that the hybrid HC294, bred for heat and drought tolerance (Khanna, 1966) and the wild species *S. pheruja*, selected for a parent in breeding cultivars adapted to lowland tropics (Mendoza and Estrada, 1979), showed growth reductions of 31% and 10% respectively, while Kufri Kunda, known to be drought sensitive showed growth reduction of 79%. This indicated that the method employed by Bansal *et al.* (1991) was valid for screening potato genotypes for drought tolerance, in which Kennebec was grouped with drought tolerant varieties. In fact, Kennebec has been listed as a drought-tolerant cultivar (Barclay and Scott, 1997). Furthermore, the linear regression analysis showed that there was a significant relationship between RLDR 2001 and RLDR 2002 ($r^2 = 0.88^*$), suggesting that the results were repeatable, and hence RLDR may be used to select potato genotypes grown *in vitro* for water-stress-tolerance.

Water stress (8% PEG 8000) also caused severe reduction in root dry weight (RDWR) for some genotypes, but a much smaller reduction for Kennebec (Table 2). The RDWR values of Kennebec were 20.4 % (2001) and 38.1% (2002), respectively, which were comparable to the results of Bansal *et al.* (1991). According to the criteria of Demagante *et al* (1995), Kennebec would be the most water-stress-tolerant cultivar tested in this experiment. The linear relationship between RDWR 2001 and RDWR 2002 was significant ($r^2 = 0.79^*$), indicating that the results were repeatable. As a consequence, the RDWR of potato genotype grown *in vitro* may be used as a trait to select potato genotypes for drought tolerance. The linear relationship between the average values of RLDR and RDWR was also significant ($r^2 = 0.86^*$), suggesting that either one can be used to select potato genotype grown *in vitro* for water stress.

Kennebec also showed the lowest shoot dry weight reduction (SDWR) in 2001 (11.7%), but was not statistically different from Tacna, Shepody, Reichie, and Andover (Table 3). The last four genotypes were also statistically equal to the rest of the genotypes. In 2002, the lowest SDWR was found in Superior, even though it was not statistically different from Unica, Kennebec, Russet Burbank, and Shepody (Table 3). There was no consistency in the ranking of the genotypes. For example, Superior showing the lowest SDWR value in 2002 and was in the 6th rank in 2001, while Andover had the highest SDWR in 2002 was the 2nd lowest in 2001. This inconsistency was further confirmed by the result of the linear regression analysis of SDWR, which was not significant over experiments ($r^2 = 0.0003^{ns}$). It was not clear what caused the inconsistency in the ranking of SDWR. Perhaps, it was attributed to the differences in the physiological age between plants used in the experiments, as mentioned in previous section (Villafranca, 1998). Another factor that might

also contribute to the inconsistency was the seasonal change, which might affect the room temperature used to incubate the plant materials. The experiment was carried out from May to July in 2001 and from September to November in 2002; and the plant materials were incubated in a room (not in the growth chamber) whose temperature was controlled by an air conditioner. Regardless of the inconsistency, the average SDWR of Kennebec was comparable to the growth reduction of leaf disc reported by Bansal *et al.* (1981), confirming that Kennebec was tolerant to water stress. Furthermore, the linear correlation between RDWR and SDWR was significant ($r^2 = 0.50^*$), with moderate relationship suggesting that both RDWR and SDWR should be used simultaneously should some one to use them for *in vitro* screening.

Kennebec demonstrated a significant increase in root-to-shoot ratio (RS), shown by the negative values of RS reduction (RSR), -120% in 2001 and -18% in 2002 (Table 3). Along with Kennebec, C89.315, and Tacna in 2001 and Reichie and E86.001 in 2002, also demonstrated an increase in RS when exposed to water stress, in one of the two years. On the other hand, the rest of the genotypes demonstrated a significant reduction in root-to-shoot ratio (RSR). The reduction in root-to-shoot (RS) was contradictory to the general knowledge, in that plants tend to increase their RS when exposed to water stress (Struik and Voorst, 1986; Jefferies, 1993). In addition to the age factor as previously mentioned, this discrepancy might also be attributed to the differences in the environmental conditions where the plants were grown. One should keep in mind that the tendency of plants to increase the RS by allocating more assimilates to the roots when exposed to water stress is the normal response of plants grown in the field (Struik and Voorst, 1986). This enhances their ability to explore deeper soil layers to extract more water (Gardner *et al.*, 1991). In this experiment, however, plants were grown *in vitro* (very humid) and supplied with sugar, vitamins and minerals (Zhang and Donnelly, 1994). Therefore, the demand for water by the shoots would have been much lower than for field's grown plants.

The ranking of RSR was not consistent over the years and the linear correlation between RSR 2001 and RSR 2002 was not significant ($r^2 = 0.15^{ns}$), suggesting that RSR might not be consistent enough to screen potato genotypes grown *in vitro* for drought tolerance. However, Kennebec known to be drought tolerant in a previous study (Bansal *et al.*, 1981) consistently demonstrated the characteristics of a drought-tolerant variety over the experiments in this study, while Superior known to be responsive to irrigation (Opena and Porter, 1999) showed the characteristic of a drought-sensitive variety with RSR of 72.7%. These suggested that RSR could be used to evaluate potato genotypes grown *in vitro* for drought tolerance, probably in accordance with other techniques to verify the results. The fact that the CIP genotypes demonstrated the tendency to be in the top of the groups (Table 3) supported the claim.

CONCLUSIONS

This experiment confirmed previous finding that PEG8000 was able to mimic the effect of water stress on potato crops. When exposed to water stress (8% PEG8000), Kennebec consistently demonstrated the lowest reduction in growth over the years, as measured in RLDR, RDWR, SDWR, and RSR, because of which it was considered to be the most drought-tolerant genotype.

The evidence indicated that RLDR and RDWR could be used to select potato genotypes grown *in vitro* for drought tolerance, while SDWR and RSR might or might not be used to select potato genotype grown *in vitro* for drought tolerance because of their inconsistency.

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