Open Agriculture. 2017; 2: 308–316 DE GRUYTER OPEN

Research Article Open Access Dandena Gelmesa*, Nigussie Dechassa, Wassu Mohammed, Endale Gebre, Philippe Monneveux, Christin Bündig, Traud Winkelmann

In vitro screening of potato genotypes for osmotic stress tolerance

DOI 10.1515/opag-2017-0035
Received November 12, 2016; accepted May 22, 2017

Abstract: Potato (*Solanum tuberosum* L.) is a cool season crop which is susceptible to both drought and heat stresses. Lack of suitable varieties of the crop adapted to drought-prone areas of the lowland tropics deprives farmers living in such areas the opportunity to produce and use the crop as a source of food and income. As a step towards developing such varieties, the present research was conducted to evaluate different potato genotypes for osmotic stress tolerance under *in vitro* conditions and identify drought tolerant genotypes for future field evaluation. The experiment was carried out at the Leibniz University of Hannover, Germany, by inducing osmotic stress using sorbitol at two concentrations (0.1 and 0.2 M) in the culture medium. A total of 43 genotypes collected from different sources (27 advanced clones from CIP, nine improved varieties, and seven farmers' cultivars) were used in a completely randomized design with four replications in two rounds. Data were collected on root and shoot growth. The results revealed that the main effects of genotype, sorbitol treatment, and their interactions significantly ($P < 0.01$) influenced root and shoot growth-related traits. Under osmotic stress, all the measured root and shoot growth traits were significantly correlated. The dendrogram obtained from the unweighted pair group method with arithmetic mean allowed grouping of the genotypes into tolerant, moderately tolerant, and susceptible ones to a sorbitol concentration of 0.2 M in the culture medium. Five advanced clones (CIP304350.100, CIP304405.47, CIP392745.7, CIP388676.1, and CIP388615.22) produced shoots and rooted earlier than all other genotypes, with higher root numbers, root length, shoot and root mass under osmotic stress conditions induced by sorbitol. Some of these genotypes had been previously identified as drought-tolerant under field conditions, suggesting the capacity of the *in vitro* evaluation method to predict drought stress tolerant genotypes. Most of the genotypes collected from Ethiopia were found to be susceptible to osmotic stress, except one farmers' cultivar (Dadafa) and two improved varieties (Zemen and Belete). Field evaluation of the tested materials under drought conditions would confirm the capacity of osmotic stress tolerant genotypes to perform well under drought-prone conditions and the potential interest of *in vitro* evaluation as a pre-screening component in potato breeding programs.

Keywords: drought stress; *Solanum tuberosum*; sorbitol; water deficiency

1 Introduction

Potato (*Solanum tuberosum* L.) is one of the most important tuber crops in the world (Albiski et al. 2012) and is a critical crop in terms of food security (Birch et al. 2012). The crop is an essential source of starch, antioxidants, protein, vitamins, macro and micronutrients, polyphenols, carotenoids and tocopherols in the human diet (Brown 2005). Potato plays an increasing role in the livelihood of people as a cash and food security crop in Eastern Africa (CIP 2011). In Ethiopia, the demand for potato is increasing because of urbanization and changes in consumption patterns of the urban population towards processed products like chips (Tesfaye et al. 2010). However, 70% of the total area in Ethiopia is known to be susceptible to drought

***Corresponding author: Dandena Gelmesa,** School of Plant Sciences, Haramaya University, Dire Dawa, Ethiopia,
E-mail: dandenagalmesa@gmail.com
Nigussie Dechassa, Wassu Mohammed, School of Plant Sciences, Haramaya University, Dire Dawa, Ethiopia
Endale Gebre, Ethiopian Institute of Agricultural Research, Addis Ababa, Ethiopia
Philippe Monneveux, International Independent Consultant, Ilheus, Brazil
Christin Bündig, Traud Winkelmenn, Leibniz Universität Hannover, Institute of Horticultural Production Systems, Woody Plant and Propagation Physiology, Germany

and heat (Ashenafi 2011). Moreover, the area under potato production is expanding, especially in lowland areas, which are frequently prone to drought episodes. The proportion of potato area that receives sufficient rainfall only represents 31% and 9% of the highlands and lowlands, respectively. Developing varieties tolerant to drought is consequently a priority for potato breeders in Ethiopia.

Potato is a cool season crop which is susceptible to both drought and heat stresses (Iwama and Yamaguchi 2006; Monneveux et al. 2013). To grow the crop at lower altitudes, developing new varieties with special adaptation to hot and dry climates is important. To this effect, there is an increasing interest in exploring and exploiting the wide genetic variation that exists for abiotic stress tolerance in farmers' cultivars and native potatoes. Breeding objectives of the International Potato Center (CIP) include drought and heat tolerance together with high yield, earliness, adaptation to long photoperiod, processing and tuber nutrient quality, and resistance to viruses and late blight (Cabello et al. 2012; Raymundo et al. 2014). In Ethiopia, developing potato varieties tolerant to drought would not only ensure a sustainable production in the country but also make it more resilient to drought stress, as part of climate adaptation strategy. Even if a potato is considered as a drought susceptible crop, some genotypes perform better than others under drought-prone conditions (Arvin and Donnelly 2008; Vasquez-Robinet et al. 2008; Bündig et al. 2016a). In line with this, CIP has developed advanced potato breeding clones tolerant to drought and/or heat stresses and reported promising results. Those genotypes could become a valuable asset to enable the production of the crop in the lowland abiotic stress areas of Ethiopia where potato varieties previously released in the country are not productive.

In vitro screening of a large number of genotypes for osmotic stress can be carried out by adding sorbitol to the Murashige and Skoog (MS) medium to reduce the osmotic potential (Murashige and Skoog 1962). Such assays can identify genotypes based on osmotic stress tolerance, are less costly, less time consuming than field trials, and easier to reproduce (Gopal and Iwama 2007). Hence, the objective of this experiment was to identify genotypes with tolerance to osmotic stress, with the assumption that they will perform well under drought conditions in the field. The genotypes included in the current experiments were collected from different sources, including CIP advanced clones selected for drought tolerance, improved varieties developed in the country, and local cultivars selected and produced by farmers (farmers' cultivars).

2 Materials and Methods

2.1 Plant materials

Forty-three potato genotypes were used in the present experiment (Table 1). The genotypes included 27 CIP advanced clones (some of them have been reported for drought or heat tolerance or a combination of both traits), nine improved varieties released in Ethiopia for production in highland areas, and seven farmers' cultivars popularly grown in eastern Ethiopia.

2.2 Experimental conditions

The genotypes were first cultivated in pots under greenhouse conditions in Ethiopia. Explants from each genotype were grown *in vitro* in test tubes containing 20 ml of solid Murashige and Skoog (1962) medium, supplemented with 30 g l^{-1} sucrose and 7.5 g l^{-1} plant agar. *In vitro* propagated genotypes were sent to the Leibniz University of Hannover and further multiplied in tissue culture to provide sufficient plantlets for the osmotic screening experiment. The multiplied genotypes were maintained *in vitro* for two to three weeks before being used in the experiment. Then, 1.5-2.0 cm long stem cuttings with one or two axillary buds were prepared by excluding the basal and apical portions of the plantlets, to be evaluated for osmotic stress tolerance. Three osmotic stress treatments were used for screening of the genotypes. Treatment one was a control without sorbitol, while treatment two and three represented osmotic stress conditions induced by the addition of 0.1 and 0.2 M sorbitol, respectively, in the culture medium, supplemented with 30 g l^{-1} sucrose and 7.5 g l^{-1} plant agar (Duchefa Biochemie B.V., Haarlem, The Netherlands). Water potential (Ψ_w) in the culture medium of the three treatments was expected to be -0.8 (control), -1.1 (mild stress) and -1.35 (strong stress) MPa, respectively, according to previous authors (Gopal and Iwama 2007). The pH of the medium was adjusted to 5.8 before autoclaving for 20 min at 120°C. Five explants of each genotype were grown in a vessel with approximately 80 ml of the growing medium in 500 ml plastic vessels. The experiment was laid out as a randomized complete design in a factorial arrangement and replicated four times in two rounds. A total of 40 explants in eight vessels were considered for evaluation. The experimental materials were cultured for 30 days at a constant temperature of 18 ± 2°C, 16/8 hours light/dark photoperiod with a

Table 1: List of potato genotypes used for *in vitro* screening to osmotic stress tolerance using sorbitol

Genotype name	Pedigree	Traits reported	Source
CIP302499.30	720139 x 392820.1	VR, HT	CIPHQ
CIP303381.106	388611.22 x 676008	VR, HT	CIPHQ
CIP303381.30	388611.22 x 676008	VR, DT	CIPHQ
CIP304350.100	CHIEFTAIN x 392820.1	VR, DT, HT	CIPHQ
CIP304350.18	CHIEFTAIN x 392820.1	VR, DT	CIPHQ
CIP304366.46	392823.4 x 676008	VR, DT	CIPHQ
CIP304368.46	391846.5 x 676008	VR, HT	CIPHQ
CIP304371.20	MONALISA x 92.187	VR, DT, HT	CIPHQ
CIP304371.67	MONALISA x 92.187	VR, DT, HT	CIPHQ
CIP304383.80	800824 x 92.187	VR	CIPHQ
CIP304387.39	REINHORT x 92.187	VR	CIPHQ
CIP 304394.56	SHEPOOY x 391207.2	VR	CIPHQ
CIP304405.42	WA.018 x 676008	VR	CIPHQ
CIP304405.47	WA.018 x 676008	VR, HT	CIPHQ
CIP304406.31	WA.077 x 676008	VR, DT, HT	CIPHQ
CIP388615.22	B-71-240.2 x 386614.16	VR	CIPHQ
CIP388676.1	378015.18 x PVY-BK	VR	CIPHQ
CIP388972.22	386316.1 x 377964.5	VR	CIPHQ
CIP390478.9	720087 x 386287.1	VR	CIPHQ
CIP392745.7	88078 x 386316.1	VR	CIPHQ
CIP395436.8	388615.22 x 388615.22	VR, DT	CIPHQ
CIP396311.1	391925.2 x C92.030	VR	CIPHQ
CIP397006.18	389468.3 x 88.052	VR, DT	CIPHQ
CIP397016.7	92.119 x 88.108	VR	CIPHQ
CIP397036.7	392011.1 x 392745.7	VR, DT	CIPHQ
CIP397077.16	392025.7 x 392820.1	VR, DT	CIPHQ
CIP397079.6	386768.10 x 392820.1	VR, DT	CIPHQ
Bubu	-	Improved variety	HU
Belete	-	Improved variety	HARC
Bulle	-	Improved variety	AwARC
Chala	-	Improved variety	HU
Gorebella	-	Improved variety	ShARC
Jalene	-	Improved variety	HARC
Zemen	-	Improved variety	HU
Chiro	-	Improved variety	HU
Gudane	-	Improved variety	HARC
Bate	-	Farmers' cultivar	RHSPC
Dadafa	-	Farmers' cultivar	RHSPC
Jarso	-	Farmers' cultivar	RHSPC
Local-Chiro	-	Farmers' cultivar	RHSPC
Tulema	-	Farmers' cultivar	RHSPC
Samune	-	Farmers' cultivar	AJIUC
Matahara	-	Farmers' cultivar	BMC

VR-virus resistant; DT-drought tolerant; HT-heat tolerant; CIPHQ-International Potato Center Head Quarter; HU-Haramaya University; HARC-Holetta Agricultural Research Center; AwARC-Awassa Agricultural Research Center; ShARC-Sheno Agricultural Research Center; RHSPC-Rare Hora Seed Producer Cooperative; HFSPC-Haji Faji Seed Producer Cooperative; AJIUC-Abdi Jalela Irrigation User Cooperative; BMC-Bilisa Multipurpose Cooperative.

photosynthetically active photon flux density of approx 35 µmol m^{-2} s^{-1} before measurement of root and shoot related morphological traits.

2.3 Measurement of traits

Days to shoot and root formation were recorded by counting the number of days from the beginning of the *in vitro* culture to when 75% of the plantlets started to produce primary shoots and roots. Shoot height (cm) was measured as the length of the main stem from the base to the tip of the plantlet after carefully removing the shoot part from the root part. Leaf number per plantlet was assessed by counting the total number of new leaves produced from the plantlet. Root number per plantlet was recorded after carefully removing the plantlet from the growing medium, separating agar and counting the total number of roots (all types of roots) produced at approximately one cm from the basal tip of the plantlet. Root length (cm) was measured by considering the maximum length of the root produced per plantlet. Shoot fresh mass (mg per plantlet) was measured by weighing shoot parts of the plantlets using a sensitive balance. Root fresh mass (mg per plantlet) was assessed after carefully removing the root portion of the plantlets from the growing medium and cleaning all agar traces using tissue paper. Shoot and root dry mass (mg per plantlet) was recorded after drying fresh samples in a drying cabinet (oven) at 70°C for 72 hours according to the procedure suggested by Schafleitner et al. (2007).

2.4 Statistical analysis

The data was subjected to analysis of variance (ANOVA) using SAS version 9.2 computer software. Statistical difference between means of the morphological traits due to genotype and treatment effects and their interactions were tested. Pearson correlation coefficients were calculated among all traits at 0 (control) and 0.2 M sorbitol concentrations using IBM SPSS statistics 24. The unweighted pair group method with arithmetic mean of cluster analysis was used at 0.2 M sorbitol concentration to construct the dendrogram of the 43 potato genotypes, based on the Euclidean distance matrix using IBM SPSS statistics 24.

3 Results

Genotype, treatment effects, and their interactions were significant ($P < 0.01$) for all morphological traits assessed in the study (Table 2). Growth reduction, as well as delays in rooting and shooting, were observed for all genotypes as the sorbitol concentration of the culture medium increased to 0.2 M (Table 3). Under no sorbitol treatment, the time to rooting and shooting of the genotypes was in the range of 5 to 14 and 5 to 13 days, respectively. However, as the sorbitol concentration increased in the culture medium, the time taken for rooting and shooting of the genotypes increased in the range of 8 to 22 and 8 to 25 days, respectively. The traits most affected by the osmotic stress treatments were shoot height (-61.0%), root number (-51.4%), and shoot dry mass (-42.9%) and the ones less affected were root

Table 2: Mean square value of osmotic stress related traits of potato genotypes as affected by genotype, sorbitol treatments and their interactions

Source of variation	DF	Mean square										
		DRF	DSF	LN	SH	SFM	RFM	RL	RN	SDM	RDM	R:S (in DM)
Genotype (G)	42	57.991***	42.42***	14.117***	12.796***	18857.096***	6685.300***	41.885***	83.596***	71.299***	71.859***	0.578***
Treatment (T)	2	2674.053***	3056.12***	310.140***	524.735***	907964.584***	81443.510***	849.035***	298.278***	1244.254***	435.198***	0.143***
GXT	84	11.442***	10.05***	2.543***	2.526***	4915.430***	425.929***	5.403***	12.391***	14.209***	3.687***	0.037***
Error	387	1.474	1.615	0.472	0.449	382.582	133.51	1.186	2.343	3.768	1.180	0.011
CV (%)		10.61	11.19	12.55	23.16	21.12	19.97	15.64	22.59	22.74	17.45	22.07
R^2		0.87	0.86	0.77	0.82	0.89	0.80	0.79	0.71	0.78	0.80	0.74

DF-degree of freedom; DRF-days to root formation; DSF-days to shoot formation; LN-leaf number per plantlet; SH-shoot height (cm); SFM-shoot fresh mass (mg per plantlet); RFM-root fresh mass (mg per plantlet); RL-root length (cm); RN-root number per plantlet; SDM-shoot dry mass (mg per plantlet); RDM-root dry mass (mg per plantlet); R:S-root to shoot ratio; DM-dry mass; CV-coefficient of variation; R^2-coefficent of determination.

Table 3: Mean, standard deviation of the mean±(SD(m)), range, F-test and variance (σ^2) of morphological growth traits of potato genotypes grown *in vitro*

Traits	Control (no sorbitol)				0.1 M sorbitol				0.2 M sorbitol			
	Mean±SD	Range	σ^2	F-test	Mean±SD	Range	σ^2	F-test	Mean±SD	Range	σ^2	F-test
DRF	8.8±1.5	5.0 -14.0	1.32	***	11.1±2.17	7.0-24.0	3.14	***	14.4±2.8	8.0-22.0	4.22	***
DSF	8.6±1.3	5.0 -13.0	1.5	***	10.9±1.7	7.0-15.0	3.56	***	14.5±2.7	8.0-25.0	3.83	***
LN	6.3±0.8	4.17-8.8	0.79	***	5.7±0.1	2.0-8.7	1.79	***	4.5±1.4	1.0-7.6	1.52	***
SH	4.1±1.3	1.6-8.2	1.09	***	2.9±1.1	1.0-7.3	0.76	***	1.6±0.7	0.2-4.6	0.28	***
RL	8.6±1.9	2.9-12.7	2.74	***	6.9±1.7	1.2-12.6	2.38	***	5.4±1.6	0.5-9.6	0.98	***
RN	11.1±3.7	4.8-27.5	3.45	***	9.3±3.2	1.5-18.8	5.79	***	5.4±2.2	1.1-13.8	4.70	***
SDM	4.9±2.2	0.2-15.6	7.22	***	4.3±2.3	0.1-15.1	5.66	***	2.8±1.7	0.04-9.1	3.03	***
RDM	0.4±0.2	0.1-0.9	12.72	***	0.5±0.2	0.1-1.3	1.25	***	0.5±0.3	0.2-1.8	1.01	***
R:S	7.2±2.7	2.4-15.5	0.97	***	7.6±2.5	1.5-13.8	0.98	***	5.8±2.4	1.2-11.6	1.12	***

DRF-days to root formation; DSF-days to shoot formation; LN-leaf number per plantlet; SH-shoot height (cm); RL-root length (cm per plantlet); RN-root number per plantlet); SDM-shoot dry mass (mg per plantlet); RDM-root dry mass (mg per plantlet); R:S-root to shoot ratio; ***-significant at P < 0.001.

to shoot dry mass ratio (-19.4%), leaf number (-28.6%), and root length (-37.2%) (Table 3). Conversely, root dry mass increased with osmotic stress (+25%). The effects of osmotic stress on shoot growth differed significantly among the genotypes, as shown in Figure 1. Shoot growth in the 0.2 M sorbitol treatment compared to the control, allowed a ranking of the genotypes according to the effects of osmotic stress on this trait (Figure 2). The most affected genotypes were the farmers' cultivar Jarso and the improved variety Chiro whereas the least affected genotypes were the CIP advanced clones CIP304350-100 and CIP388676-1. Interestingly, root dry mass was not affected or even increased (+25%) under the osmotic stress of 0.2 M sorbitol as compared to the control (Table 3).

Furthermore, morphological traits measured under 0.1 M and 0.2 M sorbitol-induced osmotic stress positively and significantly correlated with each other (Table 4). The highest positive correlation coefficient was recorded

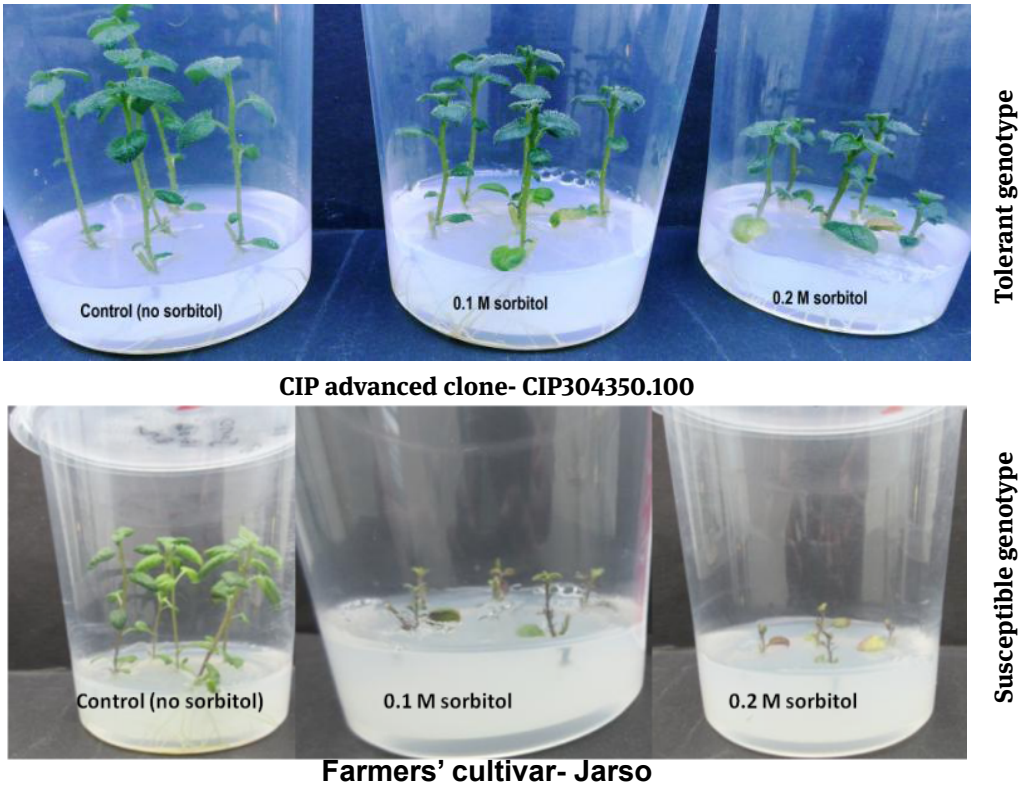

Figure 1: *In vitro* shoot growth of two potato genotypes with contrasted drought tolerance under three osmotic treatments (0, 0.1 and 0.2 M sorbitol) conditions at 30 days of culturing in growing medium

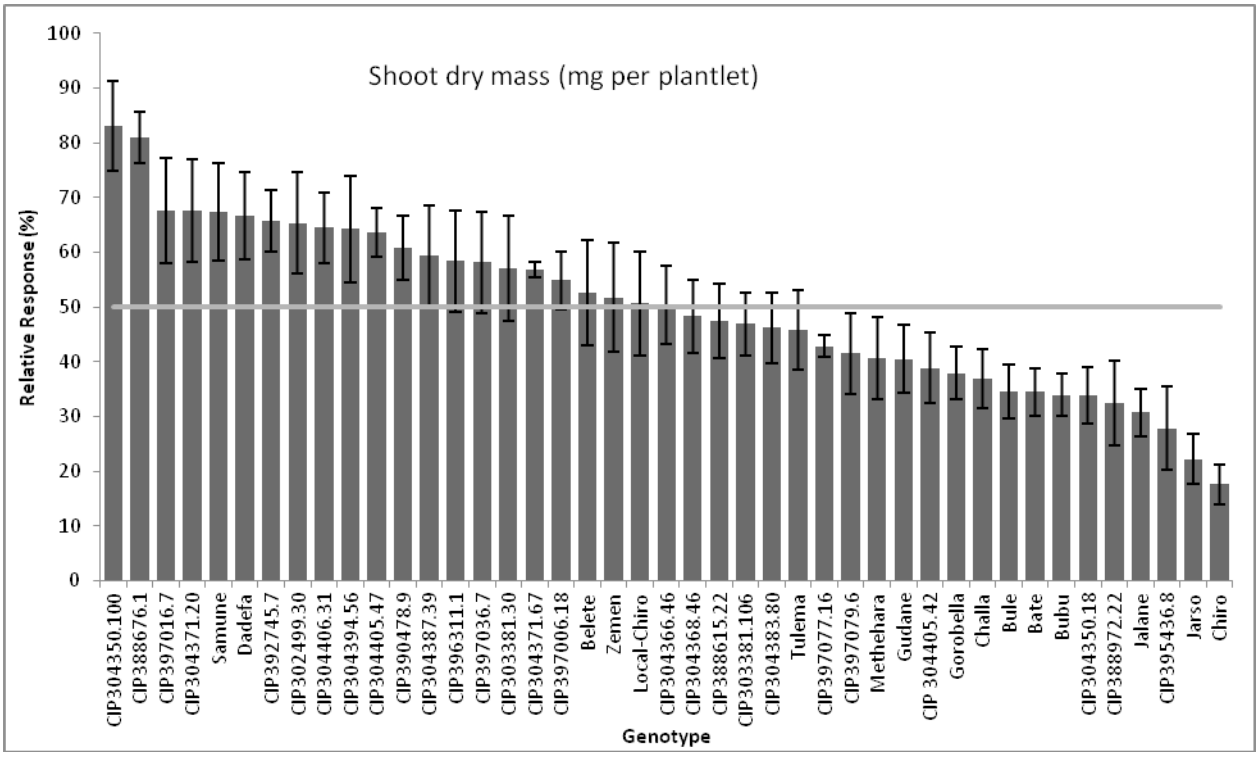

Figure 2: Shoot dry mass relative response of the 43 potato genotypes in the 0.2 M sorbitol treatment, compared to the control

Table 4: Pearson correlation coefficients between morphological traits under 0.2 sorbitol induced osmotic stress-environment of potato genotypes grown *in vitro*

Treatments		Moisture stress induced by 0.2 M sorbitol								
	Traits	**Leaf number**	**Shoot height**	**Shoot fresh mass**	**Root fresh mass**	**Root length**	**Root number**	**Shoot dry mass**	**Root dry mass**	**R:S (in dry mass)**
Control	Leaf number	0.407***	0.214**	0.367***	0.151ns	0.099ns	0.340***	0.398***	0.238**	-0.106ns
	Shoot height	0.294***	0.527***	0.329***	0.239**	0.218**	0.238**	0.299***	0.196*	-0.039ns
	Shoot fresh mass	0.200**	0.270***	0.401***	0.113ns	0.148ns	0.235**	0.340***	0.098ns	-0.140ns
	Root fresh mass	0.601***	0.614***	0.612***	0.614***	0.515***	0.545***	0.529***	0.581***	0.336***
	Root length	0.280***	0.182*	0.189*	0.300***	0.497***	0.202**	0.196*	0.371***	0.389***
	Root number	0.371***	0.328***	0.493***	0.245**	0.166*	0.522***	0.482***	0.238**	-0.100ns
	Shoot dry mass	0.245**	0.280***	0.429***	0.132ns	0.196*	0.317***	0.447***	0.167*	-0.145ns
	Root dry mass	0.573***	0.551***	0.599***	0.570***	0.550***	0.578***	0.621***	0.655***	0.397***
	R:S (fresh mass)	0.323***	0.303***	0.099ns	0.389***	0.386***	0.185*	0.079ns	0.373***	0.457***
	R:S (dry mass)	0.406***	0.387***	0.265***	0.491***	0.497***	0.339***	0.252**	0.532***	0.610***

***, **, *- significant at P < 0.001, 0.01 and 0.05, respectively; ns-non significant at P < 0.05; R:S-root to shoot ratio.

between root fresh mass and leaf number; root fresh mass and shoot height; root fresh mass and shoot fresh mass; and root fresh mass and root fresh mass. Root dry mass also highly correlated with shoot dry mass and root dry mass.

The dendrogram obtained from the unweighted pair group method with arithmetic mean (UPGMA) allowed grouping the genotypes into three main clusters and one outlier genotype. The outlier genotype Jarso is a local potato cultivar collected from farmers' fields. Among the three other clusters, cluster-I (14% of the tested genotypes) contained six CIP advanced clones. Cluster-II included one local cultivar (Dadafa), two improved varieties (Zemen and Belete) and ten CIP advanced clones. Cluster-III contained the largest proportion (53%) of potato genotypes (five local cultivars, seven improved varieties, and 12 CIP advanced clones).

4 Discussion

Screening a large number of genotypes for drought tolerance in the field is difficult due to spatial heterogeneity of soil chemical and physical properties and seasonal fluctuations. *In vitro* screening of potato genotypes for osmotic stress tolerance has been proposed as an alternative or complementary approach to costly, labour-intensive and sometimes problematic field-based screening (Rahman et al. 2008). Gopal and Iwama (2007) and Albiski et al. (2012) indicated that osmotic stress induced chemicals such as sorbitol could help to rapidly identify a large number of potato genotypes for drought tolerance. The same authors reported that addition of sorbitol or polyethylene glycol (PEG) to the MS medium decreased the water potential of the medium, inducing water stresses that adversely affected both shoot and root growth of plantlets. In the present study, water stress induced by sorbitol significantly affected the overall growth of plantlets *in vitro*, with the exception of root dry mass. A wide variation was noted among the genotypes for all measured morphological traits, in the different treatments. The differential response of potato genotypes to osmotic stress tolerance confirmed previous results from Levy (1983), Wishart et al. (2013), and Wishart et al. (2014). Similarly, Bündig et al. (2016b) reported that a wide variation was noted among potato genotypes for root and shoot dry mass due to the treatment effects of sorbitol at 0.2 M. The increased partitioning of assimilates towards the root at the expense of the shoot growth (with negative correlations) could be an explanatory trait for the resistance to osmotic stress tolerance of promising genotypes. A common response of plants to drought and osmotic stress is a shift towards higher root growth to improve water uptake and survival (Jefferies 1995; Gedroc et al. 1996; Lloret et al. 1999; Bündig et al. 2016b).

The UPGMA cluster analysis used in this study allowed to group the genotypes according to the effects of osmotic stress on root and shoot growth of the plantlets. The outlier genotype Jarso was characterized by a thin stem and a weak vegetative growth and both root and shoot growth are the most affected by osmotic stress (Figure 1 and 2). Interestingly, this variety had been previously reported by Helen et al. (2014) as producing a high proportion of small tubers, possibly as a consequence of its susceptibility to drought. The genotypes grouped in Cluster-I had the highest growth and the shortest days to rooting and shooting under osmotic stress. Include among them are the CIP advanced clones CIP304350.100 (reported as drought and heat tolerant), CIP304405.47 (heat tolerant) and CIP304371.67 (drought and heat tolerant). The clone CIP388615.22, also included in this group, was identified as highly productive under drought-prone conditions in Uzbekistan (Carli et al. 2013). However, some CIP advanced clones not previously reported as drought tolerant are also grouped in this cluster. Growth traits of genotypes under Cluster-II were moderately affected by osmotic stress and are presumed to be moderately tolerant genotypes. Genotypes categorized under Cluster-III had the lowest growth under osmotic stress, delayed rooting and shooting characteristics, suggesting a high susceptibility to osmotic stress. Most of the Ethiopian genotypes were included in this group, except the farmers' cultivars Dadafa and the improved varieties Zemen and Belete. The latter variety, which is also late blight resistant, is appreciated by Ethiopian farmers for its productivity (Mitiku et al. 2015) and is recommended for mainly processing because of its high tuber dry matter content and yield stability (Tesfaye et al. 2013). The high level of osmotic stress tolerance of some CIP advanced clones, compared to the Ethiopian genotypes (farmers' cultivars and improved varieties) is a notable observation from this experiment. This would suggest that the CIP advanced clones should be included in future potato field trials to be conducted in both the highland and lowland regions of the country to develop drought tolerant potato varieties.

5 Conclusions

In vitro screening of potato genotypes for osmotic stress tolerance evaluated in the current study demonstrated that increased osmotic stress levels due to sorbitol treatment in the growth medium resulted in reduced shoot and root growth and delayed root and shoot formation of most of the tested potato genotypes. However, some of the genotypes were observed to have tolerance to the osmotic stress, indicating the possibility to select genotypes that could adapt to drought in field conditions. A lesser effect of osmotic stress on the growth of potato genotypes previously identified as drought tolerant in field conditions suggested that *in vitro* screening of potato genotypes for osmotic stress, using sorbitol as an osmotic agent, could be efficiently used to predict or pre-screen drought tolerant clones. As suggested by Gopal and Iwam (2007) the *in vitro* method could be particularly helpful to screen a large number of plant genotypes within a short period of time. However, the effectiveness of *in vitro* screening should be further tested under field conditions on promising potato genotypes for better root yield and quality production capacity under different moisture stress regimes. Therefore, evaluating the potato genotypes

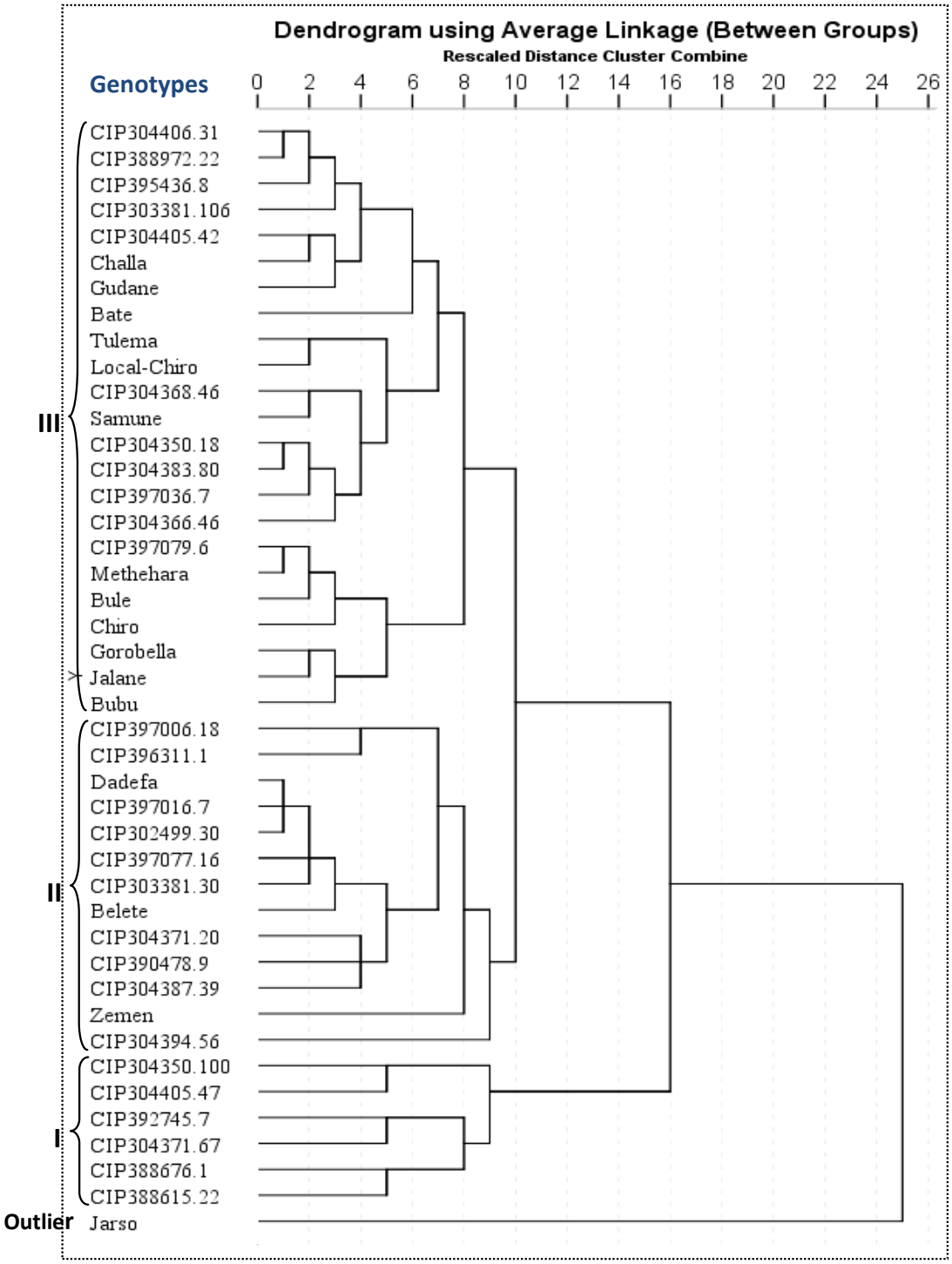

Figure 3: Dendrogram of 43 potato genotypes revealed by unweighted pair group method with arithmetic mean cluster analysis based on morphological osmotic stress related data under osmotic stress induced by 0.2 M sorbitol *in vitro*

that showed *in vitro* osmotic stress tolerance further under moisture-stressed conditions in the field may validate the result and could be a step in the direction to develop potato varieties that can be cultivated in drought-prone lowaland areas of tropics.

Acknowledgments: The authors acknowledge the potato research centers and farmers in Ethiopia and the International Potato Center for their support in collecting potato genotypes. They also thank the Deuscher Akademischer Austauschdienst (DAAD) for its financial

support and the Institute of Horticultural Production Systems, Woody Plant and Propagation Physiology of the Leibniz University of Hannover for hosting the experiment and providing laboratory facilities.

References

Albiski F., Najla S., Sanoubar R., Alkabani N., Murshed R., *In vitro* screening of potato lines for drought tolerance. Physiol. Mol. Biol. Plants, 2012, 18(4), 315-321

Arvin M.J., Donnelly D.J., Screening potato cultivars and wild species to abiotic stresses using electrolyte leakage bioassay. J. Agr. Sci. Tech., 2008, 10, 33-42

Ashenafi A.A., Modeling hydrological responses to changes in land cover and climate in Geba river basin, Northern Ethiopia, PhD thesis, Department of Earth Sciences, Freie Universität Berlin, 2011, 171 p.

Birch P.R.J., Bryan G., Fenton B., Gilroy E.M., Hein I., Jones J.T., Prashar A., Taylor M.A., Torrance L., Toth I.K., Crops that feed the world 8: Potato: are the trends of increased global production sustainable? Food Security, 2012, 4(4), 477-508

Brown C.R., Antioxidants in potato, Am. J. Potato Res., 2005, 82(2), 163-172

Bündig C., Jozefowicz A.M., Mock H-P., Winkelmann T., Proteomic analysis of two divergently responding potato genotypes (*Solanum tuberosum* L.) following osmotic stress treatment *in vitro*. J. Proteom., 2016a, 143, 227-241

Bündig C., Vu T.H., Meise P., Seddig S., Schum A., Winkelmann T., Variability in osmotic stress tolerance of starch potato genotypes (*Solanum tuberosum* L.) as revealed by an *in vitro* screening: role of proline, osmotic adjustment and drought response in pot trials, 2016b, doi:10.1111/jac.12186

Cabello R., De Mendiburu F., Bonierbale M., Monneveux P., Roca W., Chujoy E., Large scale evaluation of potato improved varieties, genetic stocks and landraces for drought tolerance. Am. J. Potato Res., 2012, 89, 400-410

Carli C., Yuldashev F., Khalikov D., Condori B., Mares V., Monneveux P., Effect of different irrigation regimes on yield, water use efficiency and quality of potato (*Solanum tuberosum* L.) in the lowlands of Tashkent, Uzbekistan. Field Crops Res., 2013, 161, 90-99

CIP, Roadmap for investment in the seed potato value chain in eastern Africa, Lima, Peru, 2011, 27 p.

Gedroc J.J., McConnaughay K.D.M., Coleman J.S., Plasticity in root/shoot partitioning: optimal. Ontogenetic orboth? Funct. Ecol., 1996, 10, 44-50

Gopal J. and Iwama K., *In vitro* screening of potato against water stress mediated through sorbitol and polyethylene glycol. Plant Cell Rep., 2007, 26, 693-700

Iwama K., Yamaguchi J., Abiotic stresses. In: Gopal J., Khurana S.M., editors. Handbook of potato production, improvement and postharvest management. New York: Food Product Press, 2006, pp. 231-278

Jefferies R.A., Physiology of crop response to drought. In: Haverkort A.J., and. MacKerron D.K.L (Eds). Potato Ecology and Modeling of Crops under Conditions Limiting Growth, Kluwer Academic Publishers, The Netherlands, 1995, 3, 61-74

Levy D., Varietal differences in the response of potatoes to repeated short periods of water stress in hot climates. 2. Tuber yield and dry matter accumulation and other tuber properties. Potato Res., 1983, 26, 315-321

Lloret F., Casanovas C., Penuelas J., Seedling survival of Mediterranean shrub land species in relation to root:shoot ratio, seed size and water and nitrogen use. Funct. Ecol., 1999, 13,210-216

Mitiku M., Shiferaw W., Tadesse A., Adaptability study of improved Irish potato (*Solanum tuberosum* L.) varieties at south Ari Woreda, Ethiopia. Agriculture, Forestry and Fisheries, 2015, 4(3), 106-108

Monneveux P., Ramirez D.A. and Pino M.T., Drought tolerance in potato (*Solanum tuberosum* L.): Can we learn from drought tolerance research in cereals? Plant Sci., 2013, 205, 76-86

Murashige T., Skoog F., A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 1962,15, 473-497

Rahman M.H., Islam R., Hossain M., Haider S.A., Differential response of potato under sodium chloride stress conditions *in vitro*. J. Biol. Sci., 2008, 16, 79-83

Raymundo R., Kleinwechter U., Asseng S., Virtual potato crop modeling: A comparison of genetic coefficients of the DSSAT-SUBSTOR potato model with breeding goals for developing countries. ZENODO, 2014, http://dx.doi.org/10.5281/zenodo.7687

Schafleitner R., Gutierrez R., Espino R., Gaudin A., Pérez J., Martínez M., Domínguez A., Tincopa L., Alvarado C., Numberto G., Bonierbale M., Field screening for variation of drought tolerance in *Solanum tuberosum* L. by agronomical, physiological and genetic analysis. Potato Res., 2007, 50, 71-85

Tesfaye A., Lemaga B., Mwakasendo J., Nzohabonayoz Z., Mutware J., Wanda K.Y., Kinyae P.M., Ortiz O., Crissman C., Theile G., Markets for fresh and frozen potato chips in the ASERECA region and the potential for regional trade: Ethiopia, Tanzania, Rwanda, Kenya, Burundi and Uganda. Social Sciences Working paper, International Potato Centre (CIP), Lima, 2010, 44 p.

Tesfaye A., Shermarl W., Thunya T., Evaluation of specific gravity of potato varieties in Ethiopia as a criterion for determining processing quality. Kasetsart J. (Nat. Sci.), 2013, 47, 30-41

Helen T., Nigussie D., Tekalign T., Assessing the yield performance of improved and local potato (*Solanum tuberosum* L.) cultivars grown in Eastern Ethiopia. Adv. Life Sci. Tech., 2014, 24, 41-48

Vasquez-Robinet C., Mane S.P., Ulanov A.V., Watkinson J.I., Stromberg V.K., De Koeyer D., *et al*., Physiological and molecular adaptations to drought in Andean potato genotypes. J. Exp. Bot., 2008, 59(8), 2109-2123

Wishart J., George T.S., Brown L.K., Ramsay G., Bradshaw J.E., White P.J., Gregory P.J., Measuring variation in potato rots in both field and glasshouse the search for useful yield predictors and a simple screen for root traits. Plant Soil, 2013, 368, 231-249

Wishart J., George T.S., Brown L.K., White P.J., Ramsay G., Jones H., Gregory P.J., Field phenotyping of potato to assess root and shoot characteristics associated with drought tolerance. Plant Soil, 2014, 378, 351-363