ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 8 (1): 132-137, 2009
ISSN 1682-296X

In vitro and *in vivo* Screening of Potato Cultivars Against Water Stress by Polyethylene Glycol and Potassium Humate

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Abstract: Plantlets produced from meristem culture of four cultivars (Agria, Savalan, Satina and Caesar) propagated by single node cuttings, in May of 2008. Experimental design was factorial on the basis of completely randomized design with two factors in three replication. Factor A was plantlets of four cultivars and factor B was four treatment (One concentrations of polyethylene glycol as -1.5 bar $1L^{-1}$ MS medium, second concentrations of potassium humate (1 mL 1L MS medium) and third concentrations of PEG (-1.5 bar) with potassium humate (1 mL 1L MS medium) and other without them as control. Chlorophyll fluorescence leaves measured by chlorophyll flurometer (OS-30p) after 30 days planting. Nitrate reductase activity (NRA) measured 30 and 40 days after planting in all of organs (leaf, stem and root). Five plantlets from each cultivar cultured in a greenhouse. The highest rate of Fv/m to Caesar under normal and normal with potassium humate and Savalan stress conditions. Consequently, cultivars were ranked in order of deficit tolerance based on reductions in Fv/Fm values. Based on Fv/Fm ranking, Agria, Caesar and Savalan cultivars were identified as water deficit tolerance. Satina and Caesar cultivars had the highest rate of NRA and Savalan and Agria the lowest in all of organs. The maximum minituber average weight, number and weight per plant for was under normal with potassium humate condition. The high value of number minituber per plant were found in Savalan and Caesar under normal, normal with potassium humate and stress with potassium humate and Caesar under stress conditions.

Key words: PEG, NRA, chlorophyll fluorescence, potassium humate, potato

INTRODUCTION

Water deficit is a common stress in potato production, which lead to decrease in tuber quality and yield. Because of potato susceptibility to drought (Hassanpanah *et al*., 2008a), preparing sufficient water is very important for increasing potato quality and quantity. It is very necessary to study about tolerance of different potato cultivars against water deficit stress and determination of potato water consumption in Ardabil. There is water deficit problem in this region. Climatic changes were occurred in Ardabil region at the recent years. These changes caused differences in precipitation dispersion, river flowing and wells water. Therefore, we have to identify agronomic characters and water need of new potato cultivars and more improvement of their quality and quantity.

Water deficit decreased number of leaves, plant water potentials (Frensch, 1997), leaf area, stem height, ground coverage, tuber number, growth and yield, canopy radiation interception and only to a lesser extent by effects on radiation use efficiency, harvest index and tuber dry matter concentration (Schittenhelma *et al*., 2006) and nitrate reductase (Chandra *et al*., 2004; Das *et al*., 2005; Kar *et al*., 2005; Xu and Guang, 2006) and increased reducing sugar content in the stem, tuber cracking and malformation, surface abrasions, hollow heart, brown center, internal brown spot, vascular discoloration or bruise, degradation of starch in the tuber stem end and total glycoalkaloids concentration (Papathanasiou *et al*., 1999). Reflectance indices were used to measure biomass and drought stress, changes in leaf water content (Francois and De Proft, 2005) and water stress (Bahrun *et al*., 2003). A set of drought tolerance genes previously found to be up-regulated in tolerant potato under drought (Schafleitner *et al*., 2007) was assayed for expression changes in potato under drought.

Potassium humate is an active hormone with natural origin that extracts from plants and animal remains exist in the bottom of marshes. This material is formed from N, P, K and microelements namely Mo, Cu, Zn, B, Co and Mg (Gadimov *et al*., 2007). Using of potassium humate increased tuber yield about 11.01 ton ha^{-1} under water stress condition (Hassanpanah *et al*., 2008a), root number (Baraldi *et al*., 1991) and seed numbers and weight in pea under saline stress condition and decreased nitrate amounts in leaves and roots (Gadimov *et al*., 2007) and in potato tubers (Hassanpanah *et al*., 2007) and decreased of plantlet transplantation from *in vitro* to greenhouse from 30 days to 13 days in MS media culture (Hassanpanah *et al*., 2008b).

Polyethylene glycol (PEG) is a polymer produced in a range of molecular weights. During the 1970's and 1980's PEG of higher molecular weight (4000 to 8000) was quite commonly used in physiological experiments to induce controlled drought stress in nutrient solution cultures. Several studies also reported theoretical or measured concentration osmotic potential relations for PEG of different molecular weights. An example for such relationship which can be roughly defined as standard calibration curves was presented by Money (1989).

Chlorophyll fluorescence is very useful to study the effects of environmental stresses on plants since photosynthesis is often reduced in plants experiencing adverse conditions, such as water deficit, temperature, nutrient deficiency, polluting agents, attack by pathogens. In condition of drought climate, shadow and deficit nutrients (the factors of decreasing of photosynthesis and protein synthesis) and the night under medium drought, but decrease in day light because of leaves plasmolysis. Certainly attraction of nitrate in condition of medium drought is more than severe drought (Scheible *et al*., 1997; Gadimov *et al*., 2007).

Crops are usually non-halophytes that tolerate only moderate salt concentrations. Nitrate reductase catalysis the first step in the nitrate-reducing pathway and is considered to be a tool to distinguish between different crop plants. Gadimov *et al*. (2007) were conducted in order to comparison of nitrate reductase activity in plants from different seasonal crops (barley, maize, soybean, cucumber and pea) under saline condition. They reported increase of salt level decreased nitrate reductase activity in all of organs and arrangement for tolerance of salinity pay attention to nitrate reduction in under study plants was barley, maize and soybean.

This experiment was conducted for evaluation of effect of polyethylene glycol and potassium humate on potato cultivars against water stress in *in vitro* and *in vivo* condition.

MATERIALS AND METHODS

***In vitro* condition:** This study was conducted in Biotechnology Laboratory and Greenhouse of Villkej Company in Ardabil Province, Iran in May of 2008. Plantlets produced from meristem culture of four cultivars (Agria, Savalan, Satina and Caesar) propagated by single node cuttings. Experimental design was factorial on the basis of completely randomized design with two factors in three replications. Factor A was plantlets that were produced from meristem culture of four cultivars and factor B was four treatments (One concentrations of polyethylene glycol as -1.5 bar $1L^{-1}$ MS medium, second concentrations of potassium humate (1 mL 1L MS medium) and third concentrations of PEG (-1.5 bar) with potassium humate (1 mL 1L MS medium) and other without them as control). Polyethylene glycol added by using diffusion based method (Taji *et al*., 2008).

Chlorophyll fluorescence of leaves measured by chlorophyll flurometer (OS-30p) since 30 days after planting. Nitrate reductase activity (NRA) measured 30 and 40 days after planting in all of organs (leaf, stem and root). Analysis of variance was done and means compared by LSD (Least significant difference) test by MSTATC software.

***In vivo* condition:** Five plantlet from each cultivar cultured in planting beds of pitmass (Biolan) with punce (1:1 v/v) in a greenhouse. Experimental design was factorial on the basis of randomized complete block design in three replication. The plantlets were planted with 10 cm distances between rows and 10 cm between plantlets. The plantlets were irrigated after planting by normal water. Macro and micro nutrients were used to provide for nutrition of plantlets. All of practices such as irrigation and control of weeds, pests and diseases were done regularly during growth period. Control of pests and fungus diseases were done respectively by use of 250^{cc} per ha Confidor and 400 g ha^{-1} Equation-Pro. Mini-tubers harvested after about two months. The traits such as minituber average weight, number and weight per plant were measured. Analysis of variance was done and means compared by LSD test, by MSTATC software. Linear correlation coefficient traits were done by SPSS software.

RESULTS AND DISCUSSION

In vitro condition

Chlorophyll fluorescence: Analysis of variance showed significant differences between MS media culture levels on rate of Fo, Fm and Fv/m, cultivars with rate of Fv/m and MS media culture levels ×cultivars on rate of Fo, Fm and Fv/m Fv/m.

The maximum leaf number, plantlet height, rate of Fo, Fm and Fv/m for MS media culture levels was under normal with potassium humate and normal toward other conditions. In order to, the rate of Fv/m increased by use of potassium humate in MS media culture under stress and normal conditions (Table 1).

The high value was found rate of Fv/m for Agria and Caesar cultivars (Table 2). The highest was belonged to Caesar under normal and normal with potassium humate; Savalan under stress conditions (Table 3).

Consequently, cultivars were ranked in order of deficit tolerance based on reductions in Fv/Fm values. Based on Fv/m ranking, Agria, Caesar and Savalan cultivars were identified as deficit tolerance.

Screening of cultivar by using chlorophyll fluorescence can provide a rapid means of gauging the freezing and chilling damage in bean, cucumber, cabbage, mandarin, grapefruit and lime (Smillie and Hetherington, 1983), freezing tolerance in potato and rice (Greaves and Wilson, 1987), effects of leaf age, irradiance and leaf water potential in potato leaves (Vos and Oyarzun, 1987), frost hardiness in black currant (Brennan and Jefferies, 1990), heat tolerance in fruit crops (Yamada *et al*., 1996), water stress in potato leaves induced (Basu *et al*., 1998), chilling susceptibility in rose (Hakam *et al*., 2000), photosynthetic response to elevated CO_2 in high altitude potato species (*Solanum curtilobum*) (Olivo *et al*., 2002), acute disturbance in water availability (Mateja *et al*., 2005), fusariosis seveity in tomato plants (Wagner *et al*., 2006), deficit tolerance in potato (Zanandrea *et al*., 2006) and combined effects of selenium and drought in potato (Mateja, 2008).

In this study, the leaf number, plant height and rate of Fv/m were increased by use of potassium humate under stress and normal conditions. The rate of Fv/m results can be attributed to the change in the capacity of exploitation of the photochemical energy in the dark adapted state. Higher values of Fo and lower

Table 1: Mean of measured traits under different levels of MS media culture

MS media culture levels	Leaf No.	Stem No.	Plantlet height (cm)	Chlorophyll fluorescence Fo	Fm	Fv/m	Nitrate reductase activity Leaf	Stem	Root	Mini-tubers No.	Mini-tubers weight (g)	Mini-tubers weight average
Normal	8.79a	1.08b	6.88c	70.58a	332.9b	0.787a	1.003b	0.729c	1.458b	1.43b	4.42bc	3.19a
Normal+Humate	8.50a	1.00b	8.50a	77.92a	378.5a	0.787a	1.366a	1.580a	1.736a	1.84a	5.88a	3.25a
Stress+Humate	7.38b	1.00b	7.21bc	70.58a	293.1b	0.747b	0.944b	0.911b	1.288b	1.49b	5.29ab	3.74a
Stress	5.63c	2.04a	7.50b	61.33b	236.3c	0.721c	0.777c	0.596d	1.083c	1.02c	4.08c	4.05a

Mean with the same letters in each column does not have significant difference at the 5% level of probability according to LSD. Fo: Minimal fluorescence, Fm: Maximal florescence, Fv/m: The ratio of variable fluorescence to maximal fluorescence, Normal: Without potassium humate and polyethylene glycol as control, Normal+Humate: 1 mL potassium humate in 1L MS medium, Stress: Polyethylene glycol as -1.5 bar 1L-1 MS medium, Stress+Humate: Polyethylene glycol as -1.5 bar and 1 mL potassium humate 1L-1 MS medium

Table 2: Mean of measured traits for potato cultivars plantlets

Cultivars	Leaf No.	Stem No.	Plantlet height (cm)	Chlorophyll fluorescence Fo	Fm	Fv/m	Nitrate reductase activity Leaf	Stem	Root	Mini-tubers No.	Mini-tubers weight (g)	Mini-tubers weight average
Satina	7.74ab	1.38a	10.50a	74.00a	293.3a	0.742b	2.110a	1.715a	1.860a	1.17c	2.79c	2.46b
Savalan	8.38a	1.42a	7.50b	69.42a	314.1a	0.761ab	0.510c	0.657b	1.011b	1.57ab	7.21a	4.77a
Caesar	6.84c	1.17a	6.25c	69.50a	322.01a	0.765ab	0.787b	0.768b	1.745a	1.73a	3.71c	2.30b
Agria	7.33bc	1.67a	5.83d	67.50a	311.4a	0.774a	0.638b	0.675b	0.951b	1.33bc	5.96b	4.70a

Mean with the same letters in each column does not have significant difference at the 5% level of probability according to LSD

Table 3: Mean of measured traits for MS media culture levels on potato cultivars plantlets

MS media culture levels	Cultivars	Leaf No.	Stem No.	Plantlet height (cm)	Chlorophyll fluorescence Fo	Fm	Fv/m	Nitrate reductase activity Leaf	Stem	Root	Mini-tubers No.	Mini-tubers weight (g)	Mini-tubers weight average
Normal	Satina	9.0c	1.3bc	9.0d	82.0abc	343.0bcd	0.760ab	1.382c	0.717cd	2.122ab	1.17c	3.00cd	2.67def
	Savalan	6.50de	1.0c	9.0d	65.67bcde	340.0bcde	0.791a	0.578def	0.682cd	1.352de	1.40bc	5.00bc	3.38cdef
	Caesar	11.0b	1.0c	11.5b	83.67ab	406.0b	0.808a	1.473c	0.855c	1.747bcd	1.83ab	5.00bc	3.17cdef
	Agria	8.67c	1.0c	12.5a	51.00ef	242.7efg	0.789a	0.581def	0.662cd	0.6133f	1.33bc	4.67bcd	3.56cdef
Normal+	Satina	8.00cd	1.0c	7.0f	63.67cde	267.0cdef	0.762ab	3.960a	4.023a	2.140ab	1.33bc	3.33cd	2.56def
Humate	Savalan	13.0a	1.0c	10.0c	76.00bc	358.7bc	0.788a	0.435f	0.637cd	1.723bcd	2.03a	8.17a	4.05cde
	Caesar	5.00e	1.0c	4.5h	94.33a	505.0a	0.799a	0.403f	0.897c	2.392a	2.17a	4.33bcd	2.03f
	Agria	8.00cd	1.0c	8.5de	77.67abc	383.03b	0.798a	0.666def	0.762cd	0.690f	1.83ab	7.67a	4.37bcd
Stress+	Satina	9.00c	1.0c	9.0g	76.33bc	284.0cdef	0.719bc	1.895b	1.628b	1.848bc	1.17c	2.50cd	2.28ef
Humate	Savalan	8.00cd	1.0c	7.0f	82.00abc	394.7b	0.793a	0.582def	0.673cd	0.4950f	1.83ab	9.00a	5.00abc
	Caesar	6.0e	1.0c	6.0g	56.00def	234.0fgh	0.760ab	0.478ef	0.692cd	1.555cde	1.83ab	3.00cd	1.64f
	Agria	6.5de	1.0c	6.0g	68.00bcde	259.7def	0.716bc	0.820d	0.650cd	1.255cde	1.13c	6.67ab	6.06ab
Stress	Satina	5.02e	2.17ab	5.5g	74.00bc	279.0cdef	0.727bc	1.207c	0.493d	1.328de	1.00c	2.33d	2.33ef
	Savalan	6.00e	2.67a	8.0e	54.00ef	163.0gh	0.671c	0.445f	0.635cd	0.473f	1.00c	6.67ab	6.67a
	Caesar	5.35e	1.67bc	6.8f	44.00f	143.0h	0.692c	0.792de	0.630cd	1.285e	1.07c	2.50cd	2.36ef
	Agria	6.17e	1.67bc	3.0i	73.33bcd	360.0bc	0.795a	0.665def	0.628cd	1.245e	1.00c	4.83bcd	4.83abc

Mean with the same letters in each column does not have significant difference at the 5% level of probability according to LSD

Fm can indicate a higher energy dissipation in PSII antenna system (Rohacek, 2002; Zanandrea *et al.*, 2006).

Tourneux *et al.* (2003) reported that at relative water deficits below 40% potato genotypes did not show differences in Fv/m values. The high dehydration resistance of photosystem II in potato has been reported by Tourneux and Peltier (1995) and 40 days of drought in the testing environment caused sufficient stress to lower yield, but no drop in chlorophyll fluorescence.

Nitrate reductase activity (NRA): Results of analysis of variance showed significant differences between effect of stage (S), MS media culture levels (A) and cultivars (B), A×B, S×A×B, for leaf, stem and root and S×A for stem and root, S×B for leaf and root.

The normal with potassium humate toward normal and stress with potassium humate toward stress conditions produced maximum rate of nitrate reductase activity (NRA) in all of organs (leaf, stem and root). The roots had high rate of NRA toward leaf and stem organs (Table 1). Increase of water deficit stress decreased NRA in all of organs. All of organs rate of NRA decreased as a result of water stress. Potassium humate causes to increase rate of NRA under normal and stress conditions.

Satina and Caesar had the highest rate of NRA and Savalan and Agria the lowest in all of organs (leaf, stem and root). Savalan, Caesar and Agria had high rate of NRA in root organ toward leaf and stem organs (Table 2).

The highest rate of NRA was produced since 30 days after plantlets. Since, 30 days and 40 days after plantlets had high rate of NRA in roots toward leaf and stem organs. The normal with potassium humate toward normal and stress with potassium humate toward stress conditions produced maximum rate of NRA since 30 days after plantlets. The root had high rate of NRA toward leaf and stem organs. The root had the highest rate of NRA and the leaf and stem the lowest under normal, stress with potassium humate and stress conditions since 30 days after plantlets. The root had the highest rate of NRA and the leaf and stem the lowest under normal with potassium humate since 30 and 40 days after plantlets.

Satina and Caesar had the highest rate of NRA and Savalan and Agria the lowest since 30 and 40 days after plantlets. The root had the highest rate of NRA in all of cultivars and since 30 days after plantlets. Satina and Caesar had the highest rate of NRA under normal and stress in root organ. Caesar had high rate of NRA under normal with potassium humate and stress with potassium humate (Table 3).

In this study, the rate of NRA decreased in normal with potassium humate, stress with potassium humate and stress leaves, stem and root compared with normal conditions. Increase of deficit stress decreased NRA in all of organs. This is consistent with previous observations of water-stress induced losses in maximal extractable foliar NR activity in other species (Wellburn *et al.*, 1996). Brewitz *et al.* (1996) reported the rate of NRA decrease in stress leaves compare with well-water conditions in tomato crop.

The roots had high rate of NRA toward leaf and stem organs. Potassium humate causes to increase rate of NRA under normal and stress conditions. NR activity decreased under water stress in strong correlation with CO_2 assimilation. Therefore, a low NR activity decrease under drought could pinpoint drought tolerant genotypes. NR activity was reported to decrease under water stress (Das *et al.*, 2005; Kar *et al.*, 2005; Xu and Guang, 2006) and a low decrease of activity has been associated with drought tolerance (Chandra *et al.*, 2004; Das *et al.*, 2005). Nitrate reductase activity was significantly decreased in drought-exposed plants, but activity depletion was independent of yield or yield maintenance (Schafleitner *et al.*, 2007).

Gadimov *et al.* (2007) resulted that NRA is very different related to plant type. But generally leaves are the basic portion of nitrate reduction (50-90%) and roots (6-40%) have the second place in this matter. Increase of salt level decreased NRA in all of organs. Solution of 50 mM NaCl produced maximum rate of NRA reduction observed in spring barley leaves. And solution of 100 mM NaCl caused that soybean organs show the highest sensitivity to the saline stress and decreased NRA 82% relative to control. In soybean, NRA decreased 5.2 times in leaves, 2.8 times in stems and 4.7 times in roots. This reduction was in barley and maize about 1.4-2.6 times. Arrangement for tolerance of salinity pay attention to nitrate reduction in under study plants was barley, maize and soybean.

***In vivo* condition:** Analysis of variance showed that was significant differences between MS media culture levels on mini-tuber number and weight per plant, cultivars on mini-tuber weight average, number and weight per plant and MS media culture levels×cultivars on mini-tuber weight per plant and average weight mini-tuber per plant.

The maximum mini-tuber weight average, number and weight per plant for was under normal with potassium humate condition (Table 1). The maximum mini-tuber weight average, number and weight per plant for Savalan cultivar (Table 2). The high value of mini-tuber number and weight per plant were found in Savalan and Caesar under normal with potassium humate condition (Table 3).

There were significant and positive correlation between leaf number with plant height; Fo with Fv/m, Fm and mini-tuber number per plant; Fm with Fv/m and mini-tuber number and weight per plant; Fv/m with mini-tuber

Table 4: Linear correlation coefficients between different traits for MS media culture levels on potato cultivars plantlets in *in vitro* and *in vivo* conditions

Correlation coefficient	Leaf No.	Stem No.	Plant height	Fo	Fm	Fv/m	Mini-tuber No.	Mini-tuber weight	Average weight	Leaf NRA	Stem NRA	Root NRA
Leaf No.	1											
Stem No.	-0.45	1										
Plant Height (cm)	0.66*	-0.24	1									
Fo	0.26	-0.32	-0.16	1								
Fm	0.26	-0.49*	-0.05	0.91**	1							
Fv/m	0.39	-0.64*	0.25	0.54*	0.81**	1						
Number of mini-tubers	0.38	-0.51*	-0.02	0.99**	0.88**	0.83**	1					
Weight of mini-tubers	0.36	-0.61	0.22	0.43	0.69*	0.67*	0.76*	1				
Average mini-tuber weight	0.05	-0.15	0.24	-0.20	0.33	0.30	-0.41	-0.32	1			
Leaf NRA	0.17	0.31	0.06	-0.22	-0.11	0.17	-0.26	-0.26	0.92**	1		
Stem NRA	0.12	-0.15	-0.03	-0.05	-0.13	-0.08	-0.53*	-0.66*	0.47	0.42	1	
Root NRA	0.15	-0.24	-0.08	0.21	-0.08	-0.01	0.05	0.17	0.12	0.14	0.42	1

* and ** : Significant at probability levels of 5 and 1%, respectively; NRA: Nitrate Reductase Activity

number and weight per plant and mini-tuber number per plant with mini-tuber weight per plant and stem number with Fm and Fv/m; negative correlation (Table 4).

CONCLUSION

Chlorophyll fluorescence measurement is a suitable tool of study changes in the photosynthetic capacity of the plants exposed to limit water of supply. Cultivars responded differently to disturbance in water deficit. Further analysis and more measurements are needed to be able to range cultivars of potato according to their different sensitivity to acute limitation of water supply (Mateja *et al*., 2005). In this study, the leaf number, plant height and rate of Fv/m increased by use of potassium humate under stress and normal conditions. The rate of Fv/Fm results can be attributed to change in the capacity of exploitation of the photochemical energy in the dark adapted state. Higher values of Fo and lower Fm can indicate a higher energy dissipation in PSII antenna system (Zanandrea *et al*., 2006).

The cultivars were ranked in order of deficit tolerance based on reductions in rate of Fv/Fm values. Based on Fv/Fm ranking, Agria, Caesar and Savalan cultivars was identified as deficit tolerance and Satina cultivar as deficit intermediate tolerant.

Satina and Caesar had the highest rate of NRA and Savalan and Agria the lowest in all of organs (leaf, stem and root). Satina and Caesar had the highest rate of NRA under normal and stress in root organ. Caesar had high rate of NRA under normal with potassium humate and stress with potassium humate.

The maximum mini-tuber weight average, number and weight per plant for were under normal with potassium humate condition and for Savalan cultivar. The high value of mini-tuber number and weight per plant were found in Savalan and Caesar under normal with potassium humate condition.

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