

Inhibition and recovery of germination and growing ability of seedlings under and after osmotic stress induced by polyethylene glycol in 8 pea genotypes

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Summary: Germination and early seedling growth of eight pea genotypes were examined under and soon after different (5%, 10%, 15%, and 20%) PEG treatments. Seeds were germinated on PEG solution for 3 or 6 days and then further germinated and cultured on filter paper moistened by water for 3 and 6 days. The length and fresh weight of shoots and roots of seedlings were measured and used for evaluation of genotypes. Roots were less inhibited by osmotic stress than shoots similarly to other plant species. The variability among the genotypes was the greatest in the case of shoot growth at 5% PEG treatment and in the case of root growth at 15% PEG treatment. Results suggest that growing responses of genotypes after cessation of stress are more suitable for the evaluation of their osmotic tolerance, than their responses expressed during *in vitro* stress conditions. Genotypes with relatively high or low osmotic stress tolerance, respectively, could be distinguished with 6 days after recovery from 3-day-long 15% PEG treatment concerning the rate of shoot weight to root weight.

Key words: osmotic stress, *in vitro*, genotype-dependence, osmoregulation

Introduction

Stress is defined by Levitt (1980) as any environmental factor, which can be potentially unfavourable to living organisms. Stresses could be biotic and abiotic ones, mild and severe stresses. Severe stress has negative effect on the plant development and damages the plant (Lichtenthaler, 1996).

One of the most important abiotic factors limiting the survival and productivity of plants is desiccation caused by decreasing environmental water potential (Sanchez et al., 1998). In Hungary the growing season is often characterized by high temperatures and few rainfalls with long drought periods, which leads to an unsatisfying water regime in soil. Therefore, improving of drought tolerance of different crops has been one of the major tasks in most breeding programmes. However, the main question involves, which simply measurable factor(s) could be used as selection criteria for drought tolerance.

Tolerance to drought is a complex phenotype in which water deficit, heat stress and oxidative stress all interact to potentiate one another and in which different traits are involved, such as impermeable cuticle, low rate of shoot to root, leaf colour, osmotic adjustment and turgor maintenance etc. (Kpoghomou et al., 1990, Sanchez et al., 2004)

Maintenance of cell turgor pressure is important for many metabolic processes and for growth (Morgan, 1984). Osmoregulation helps in the maintenance of turgor pressure under water stress conditions and therefore one of the most important mechanisms for overcoming drought (Mullet & Whitsitt, 1996).

In this paper we studied the rate of inhibition of germination and growing ability of 8 pea genotypes during and recovery from water stress induced by polyethylene glycol in order (i) to determine the differences between the different genotypes and (ii) to examine if this germination test may be used to measure osmoregulation of different genotypes, as one of the selection criteria for drought tolerance in peas.

Materials and methods

Germination and early growth of seedlings were examined under and soon after osmotic stress conditions in eight pea genotypes: 'Graphis', 'Erbi', 'Janus', 'Hanka', 'Baccara', '2107', 'Hunor', 'M49060'. Water stress was induced by PEG 600. Seeds were washed by tap water supplemented with 1–2 drops 1–1 detergent (Ultra-Sol) and then surface sterilized with 10 % Clorox (commercial

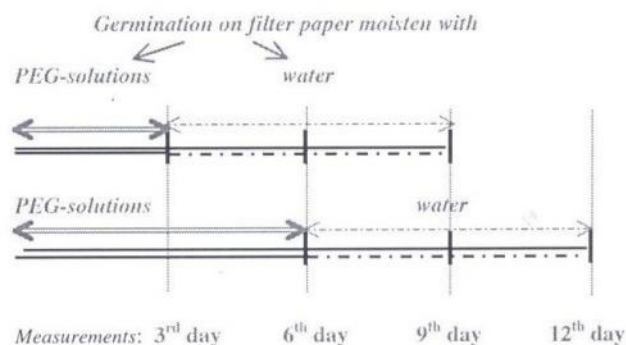


Figure 1. The applied treatments in the germination test

Table 1. Relative growth of root length (RL) (mm) subjected to osmotic stress for 3 or 6 days and with 3 or 6 days after recovery from osmotic stress in 8 pea genotypes. *

Genotype	Treatment	PEG 600				
		0 %	5 %	10 %	15 %	20 %
Baccara	3 days on PEG	100	59.45 ab	13.42 a	0 a	0 a
	6 days on PEG	100	74.24 bc	31.52 abc	7.20 bc	0 a
	3 days after recovery from 3-day-long PEG	100	77.34 c	52.58 c	44.22 c	37.89 bc
	3 days after recovery from 6-day-long PEG	100	70.91 bc	52.16 a	33.94 b	20.97 a
	6 days after recovery from 3-day-long PEG	100	77.85 cd	71.84 a	70.26 b	70.40 b
	6 days after recovery from 6-day-long PEG	100	71.59 a	69.25 a	58.80 a	55.65 a
Erbi	3 days on PEG	100	54.24 a	12.32 a	0 a	0 a
	6 days on PEG	100	60.07 a	34.16 abc	3.01 a	0 a
	3 days after recovery from 3-day-long PEG	100	58.50 a	48.17 bc	35.40 a	34.49 ab
	3 days after recovery from 6-day-long PEG	100	67.27 ab	53.00 a	26.51 a	23.80 ab
	6 days after recovery from 3-day-long PEG	100	68.73 b	70.40 a	61.71 a	64.16 a
	6 days after recovery from 6-day-long PEG	100	69.38 a	73.69 ab	65.89 bc	64.20 b
Graphis	3 days on PEG	100	70.86 cd	20.63 bc	0 a	0 a
	6 days on PEG	100	59.32 a	29.75 ab	4.31 ab	0 a
	3 days after recovery from 3-day-long PEG	100	61.12 a	53.71 c	42.18 b	37.99 bc
	3 days after recovery from 6-day-long PEG	100	70.49 bc	54.46 a	33.78 b	28.01 c
	6 days after recovery from 3-day-long PEG	100	56.44 a	71.62 a	68.06 b	71.18 b
	6 days after recovery from 6-day-long PEG	100	78.42 b	80.03 cd	72.29 d	76.70 d
Hanka	3 days on PEG	100	62.48 abc	20.13 bc	0 a	0 a
	6 days on PEG	100	79.12 cd	39.16 bc	7.09 bc	0 a
	3 days after recovery from 3-day-long PEG	100	81.28 c	44.95 b	34.98 a	35.99 b
	3 days after recovery from 6-day-long PEG	100	79.14 d	59.13 b	35.87 b	22.23 a
	6 days after recovery from 3-day-long PEG	100	80.22 d	70.44 a	66.74 ab	70.04 b
	6 days after recovery from 6-day-long PEG	100	81.96 b	78.66 bcd	79.11 e	69.61 bc
Hunor	3 days on PEG	100	74.14d	14.74 ab	3.05 c	0 a
	6 days on PEG	100	69.45 b	34.58 abc	14.69 d	0.99 b
	3 days after recovery from 3-day-long PEG	100	68.12 b	31.41 a	33.43 a	30.92 a
	3 days after recovery from 6-day-long PEG	100	63.75 a	53.80 a	41.23 d	27.50 bc
	6 days after recovery from 3-day-long PEG	100	74.92 cd	65.06 a	66.18 ab	62.40 a
	6 days after recovery from 6-day-long PEG	100	78.54 b	79.17 bcd	69.89 cd	69.81 c
Janus	3 days on PEG	100	67.32 bcd	25.07 cd	1.68 b	0 a
	6 days on PEG	100	71.43 bc	41.17 e	10.14 c	0 a
	3 days after recovery from 3-day-long PEG	100	63.83 ab	44.98 b	32.08 a	36.23 b
	3 days after recovery from 6-day-long PEG	100	75.49 cd	61.54 bc	40.22 cd	22.19 a
	6 days after recovery from 3-day-long PEG	100	73.76 c	70.92 a	68.46 b	61.57 a
	6 days after recovery from 6-day-long PEG	100	79.42 b	78.96 bcd	77.74 e	67.01 bc
M49060	3 days on PEG	100	71.11 cd	21.23 e	0.77 ab	0 a
	6 days on PEG	100	84.24 d	81.29 d	25.04 e	0.29 a
	3 days after recovery from 3-day-long PEG	100	96.86 d	75.26 d	50.11 e	44.48 d
	3 days after recovery from 6-day-long PEG	100	74.92 cd	72.55 d	46.85 e	26.44 bc
	6 days after recovery from 3-day-long PEG	100	91.15 e	87.48 b	78.50 e	73.82 b
	6 days after recovery from 6-day-long PEG	100	87.71 c	74.93 bc	64.20 b	66.64 bc
2107	3 days on PEG	100	70.70 cd	27.88 d	0.70 ab	0 a
	6 days on PEG	100	76.04 bcd	28.46 a	9.40 c	0.17 a
	3 days after recovery from 3-day-long PEG	100	81.18 c	54.43 c	40.45 b	40.66 cd
	3 days after recovery from 6-day-long PEG	100	80.05 d	64.29 c	45.15 de	28.38 c
	6 days after recovery from 3-day-long PEG	100	86.36 e	81.86 b	78.38 e	79.63 c
	6 days after recovery from 6-day-long PEG	100	83.22 bc	83.07 d	81.38 e	77.41 d

*: Letters in columns mean the differences between the genotypes in each treatment ($p < 0.05$).

Table 2. Relative growth of root weight (RW) (mg) subjected to osmotic stress for 3 or 6 days and with 3 or 6 days after recovery from osmotic stress in 8 pea genotypes. *

Genotype	Treatment	PEG 600				
		0 %	5 %	10 %	15 %	20 %
Baccara	3 days on PEG	100	91.13 d	18.47 a	0 a	0 a
	6 days on PEG	100	69.61 ab	29.40 ab	9.55 bc	0 a
	3 days after recovery from 3-day-long PEG	100	92.08 bc	65.72 b	62.09 b	51.19 ab
	3 days after recovery from 6-day-long PEG	100	85.21 ab	74.82 abc	50.57 bcd	33.31 bc
	6 days after recovery from 3-day-long PEG	100	92.37 ab	75.40 a	78.86 b	81.57 cd
	6 days after recovery from 6-day-long PEG	100	90.13 a	247.10 a	84.68 bc	69.52 a
Erbi	3 days on PEG	100	73.31 bc	15.02 a	0 a	0 a
	6 days on PEG	100	63.57 a	30.1 ab	5.31 a	0 a
	3 days after recovery from 3-day-long PEG	100	80.68 a	65.77 b	54.79 a	49.95 ab
	3 days after recovery from 6-day-long PEG	100	85.59 ab	70.84 ab	38.6 a	39.51 e
	6 days after recovery from 3-day-long PEG	100	101.54 bc	93.28 b	79.88 bc	81.99 cd
	6 days after recovery from 6-day-long PEG	100	96.77 ab	96.99 a	76.87 a	71.24 a
Graphis	3 days on PEG	100	81.78 c	29.51 b	0 a	0 a
	6 days on PEG	100	78.65 c	43.15 b	6.65 ab	0 a
	3 days after recovery from 3-day-long PEG	100	100.03 c	82.20 d	69.89 c	60.7 c
	3 days after recovery from 6-day-long PEG	100	103.64 c	78.48 bc	44.34 ab	37.87 de
	6 days after recovery from 3-day-long PEG	100	83.62 a	96.66 b	82.63 bed	76.07 bc
	6 days after recovery from 6-day-long PEG	100	118.14 cd	117.90 a	93.64 bc	92.86 c
Hanka	3 days on PEG	100	66.63 ab	29.85 bc	0 a	0 a
	6 days on PEG	100	67.85 ab	31.11 ab	11.73 cd	0 a
	3 days after recovery from 3-day-long PEG	100	90.68 b	69.96 b	55.74 a	51.36 ab
	3 days after recovery from 6-day-long PEG	100	103.49 c	74.19 abc	47.77 bc	32.97 bc
	6 days after recovery from 3-day-long PEG	100	107.46 cd	97.07 b	87.34 cd	83.49 d
	6 days after recovery from 6-day-long PEG	100	128.44 d	118.50 a	95.76 bc	81.68 b
Hunor	3 days on PEG	100	72.10 b	22.97 ab	4.73 c	0 a
	6 days on PEG	100	73.59 bc	26.35 a	17.77 f	1.64 b
	3 days after recovery from 3-day-long PEG	100	90.51 b	47.55 a	54.02 a	45.76 a
	3 days after recovery from 6-day-long PEG	100	90.36 b	67.51 a	56.87 d	34.85 cd
	6 days after recovery from 3-day-long PEG	100	107.34 cd	82.81 a	88.05 d	74.94 b
	6 days after recovery from 6-day-long PEG	100	110.52 bc	101.5 a	105.49 c	87.95 bc
Janus	3 days on PEG	100	64.72 ab	38.33 c	2.5 b	0 a
	6 days on PEG	100	78.13 c	33.18 ab	14.09 de	0 a
	3 days after recovery from 3-day-long PEG	100	92.4 bc	72.05 bc	50.73 a	52.18 b
	3 days after recovery from 6-day-long PEG	100	89.86 b	80.27 c	54.90 d	28.64 a
	6 days after recovery from 3-day-long PEG	100	112.59 d	93.20 b	86.26 bed	71.97 b
	6 days after recovery from 6-day-long PEG	100	102.93 ab	118.85 a	101.04 c	64.94 a
M49060	3 days on PEG	100	69.66 b	34.07 c	0.65 ab	0 a
	6 days on PEG	100	68.58 ab	82.3 c	24.16 g	0.4 a
	3 days after recovery from 3-day-long PEG	100	88.06 ab	76.20 cd	51.19 a	51.19 ab
	3 days after recovery from 6-day-long PEG	100	79.00 a	76.27 bc	47.75 bc	30.27 ab
	6 days after recovery from 3-day-long PEG	100	99.18 bc	78.05 a	70.99 a	64.70 a
	6 days after recovery from 6-day-long PEG	100	110.14 bc	114.00 a	97.18 c	72.00 a
2107	3 days on PEG	100	58.11 a	34.38 c	0.75 ab	0 a
	6 days on PEG	100	78.58 c	27.42 a	16.47 ef	0.40 a
	3 days after recovery from 3-day-long PEG	100	94.72 bc	78.66 cd	66.99 bc	61.63 c
	3 days after recovery from 6-day-long PEG	100	86.64 ab	76.61 bc	54.09 cd	32.23 de
	6 days after recovery from 3-day-long PEG	100	96.37 b	81.00 a	71.79 a	72.17 b
	6 days after recovery from 6-day-long PEG	100	107.17 bc	110.00 a	94.52 bc	81.82 b

*: Letters in columns mean the differences between the genotypes in each treatment ($p < 0.05$).

Table 3. Relative growth of shoot length (SL) (mm) subjected to osmotic stress for 3 or 6 days and with 3 or 6 days after recovery from osmotic stress in 8 pea genotypes. *

Genotype	Treatment	PEG 600				
		0 %	5 %	10 %	15 %	20 %
Baccara	3 days on PEG	100	0 a	0 a	0 a	0 a
	6 days on PEG	100	33.01 a	3.50 a	0 a	0 a
	3 days after recovery from 3-day-long PEG	100	72.64 a	48.31 b	30.71 ab	26.37 bc
	3 days after recovery from 6-day-long PEG	100	61.76 b	36.14 ab	22.94 de	12.28 b
	6 days after recovery from 3-day-long PEG	100	82.08 b	66.47 a	61.62 bc	59.35 c
	6 days after recovery from 6-day-long PEG	100	81.71 bc	58.64 a	52.42 ab	42.55 abc
Erbi	3 days on PEG	100	0 a	0 a	0 a	0 a
	6 days on PEG	100	37.01 ab	0 a	0 a	0 a
	3 days after recovery from 3-day-long PEG	100	74.14 a	48.8 bc	29.01 a	25.59 abc
	3 days after recovery from 6-day-long PEG	100	61.51 b	39.67 bc	15.64 a	11.62 ab
	6 days after recovery from 3-day-long PEG	100	81.59 b	71.60 a	57.15 ab	58.51 c
	6 days after recovery from 6-day-long PEG	100	76.51 ab	65.39 bc	46.72 a	45.90 cd
Graphis	3 days on PEG	100	41.84 b	0 a	0 a	0 a
	6 days on PEG	100	43.11 bcd	6.27 a	0 a	0 a
	3 days after recovery from 3-day-long PEG	100	76.05 ab	57.13 c	35.16 bc	28.86 bc
	3 days after recovery from 6-day-long PEG	100	78.65 d	42.84 c	17.74 ab	12.31 b
	6 days after recovery from 3-day-long PEG	100	62.06 a	66.02 a	55.43 ab	56.74 bc
	6 days after recovery from 6-day-long PEG	100	77.53 abc	75.82 e	54.58 bc	44.00 bed
Hanka	3 days on PEG	100	45.39 b	0 a	0 a	0 a
	6 days on PEG	100	38.17 abc	11.61 a	0 a	0 a
	3 days after recovery from 3-day-long PEG	100	89.61 c	57.18 bc	31.24 ab	28.53 bc
	3 days after recovery from 6-day-long PEG	100	63.40 bc	32.11 a	19.5 bed	11.40 ab
	6 days after recovery from 3-day-long PEG	100	79.77 b	65.51 a	56.59 ab	59.17 c
	6 days after recovery from 6-day-long PEG	100	77.89 abc	61.94 ab	51.15 ab	44.36 bed
Hunor	3 days on PEG	100	59.85 bc	0 a	0 a	0 a
	6 days on PEG	100	53.10 e	10.80 a	1.79 b	0 a
	3 days after recovery from 3-day-long PEG	100	76.25 ab	33.25 a	30.73 ab	21.43 a
	3 days after recovery from 6-day-long PEG	100	68.45 c	40.18 bc	23.96 e	12.48 b
	6 days after recovery from 3-day-long PEG	100	80.44 b	70.92 a	55.35 ab	46.99 a
	6 days after recovery from 6-day-long PEG	100	82.05 bc	72.90 de	60.02 c	45.23 bed
Janus	3 days on PEG	100	48.20 b	0 a	0 a	0 a
	6 days on PEG	100	47.77 de	10.90 a	0 a	0 a
	3 days after recovery from 3-day-long PEG	100	72.29 a	46.75 b	29.49 a	24.45 ab
	3 days after recovery from 6-day-long PEG	100	69.00 c	48.14 d	28.01 f	10.28 ab
	6 days after recovery from 3-day-long PEG	100	93.15 c	71.78 a	65.62 c	54.65 bc
	6 days after recovery from 6-day-long PEG	100	71.31 a	73.09 de	59.30 c	38.61 a
M49060	3 days on PEG	100	50.51 bc	2.96 b	0 a	0 a
	6 days on PEG	100	47.50de	72.31 b	0.37 a	0 a
	3 days after recovery from 3-day-long PEG	100	84.53 bc	66.61d	36.53 c	29.81 c
	3 days after recovery from 6-day-long PEG	100	54.37 a	42.66 c	18.9 abc	9.31 a
	6 days after recovery from 3-day-long PEG	100	81.39 b	67.43 a	54.24 a	50.84 ab
	6 days after recovery from 6-day-long PEG	100	83.61 c	70.13 cde	49.75 ab	40.45 ab
2107	3 days on PEG	100	68.75 c	0 a	0 a	0 a
	6 days on PEG	100	44.07 cd	3.75 a	0 a	0 a
	3 days after recovery from 3-day-long PEG	100	75.80 ab	52.98 bc	32.29 ab	29.01 bc
	3 days after recovery from 6-day-long PEG	100	59.27 ab	36.18 ab	22.3 cde	12.27 b
	6 days after recovery from 3-day-long PEG	100	77.91 b	63.30 a	55.62 ab	51.76 ab
	6 days after recovery from 6-day-long PEG	100	82.70 bc	69.06 cd	58.40 c	48.19 d

*: Letters in columns mean the differences between the genotypes in each treatment ($p < 0.05$).

Table 4. Relative growth of shoot weight (SW) (mg) subjected to osmotic stress for 3 or 6 days and with 3 or 6 days after recovery from osmotic stress in 8 pea genotypes. *

Genotype	Treatment	PEG 600				
		0 %	5 %	10 %	15 %	20 %
Baccara	3 days on PEG	100	0 a	0 a	0 a	0 a
	6 days on PEG	100	27.66 a	3.81 a	0 a	0 a
	3 days after recovery from 3-day-long PEG	100	70.25 a	45.23 b	30.72 a	29.11 cd
	3 days after recovery from 6-day-long PEG	100	75.77 c	60.66 a	60.82 abc	58.12 b
	6 days after recovery from 3-day-long PEG	100	53.86 b	34.03 a	22.04 b	10.31 a
	6 days after recovery from 6-day-long PEG	100	77.53 ab	62.75 a	50.74 ab	45.16 b
Erbi	3 days on PEG	100	0 a	0 a	0 a	0 a
	6 days on PEG	100	30.24 a	0 a	0 a	0 a
	3 days after recovery from 3-day-long PEG	100	68.31 a	41.82 b	32.12 a	27.11 ab
	3 days after recovery from 6-day-long PEG	100	78.66 c	68.66 ab	55.83 a	59.23 b
	6 days after recovery from 3-day-long PEG	100	54.50 b	37.03 ab	15.52 a	13.59 b
	6 days after recovery from 6-day-long PEG	100	73.54 a	65.47 a	47.98 a	48.09 b
Graphis	3 days on PEG	100	28.87 b	0 a	0 a	0 a
	6 days on PEG	100	36.78 b	5.27 a	0 a	0 a
	3 days after recovery from 3-day-long PEG	100	75.35 ab	58.87 c	35.51 ab	28.88 bcd
	3 days after recovery from 6-day-long PEG	100	58.60 b	72.30 b	58.52 ab	57.38 b
	6 days after recovery from 3-day-long PEG	100	83.90 d	40.67 bc	20.68 b	13.99 b
	6 days after recovery from 6-day-long PEG	100	76.60 ab	77.95 c	56.28 bc	47.71 b
Hanka	3 days on PEG	100	66.19 bc	0 a	0 a	0 a
	6 days on PEG	100	37.36 b	15.77 a	0 a	0 a
	3 days after recovery from 3-day-long PEG	100	88.93 c	56.51 c	35.88 ab	31.09 cd
	3 days after recovery from 6-day-long PEG	100	84.33 c	71.10 ab	68.18 bc	66.81 c
	6 days after recovery from 3-day-long PEG	100	65.44 c	38.87 abc	23.63 bc	13.69 b
	6 days after recovery from 6-day-long PEG	100	82.52 b	68.29 ab	57.57 bc	48.71 bc
Hunor	3 days on PEG	100	82.86 cd	0 a	0 a	0 a
	6 days on PEG	100	48.38 d	11.33 a	1.57 b	0 a
	3 days after recovery from 3-day-long PEG	100	71.57 ab	33.99 a	33.33 a	23.09 a
	3 days after recovery from 6-day-long PEG	100	0.075 a	65.17 ab	56.98 ab	50.12 a
	6 days after recovery from 3-day-long PEG	100	0.0061 a	37.56 ab	26.02 cd	14.03 b
	6 days after recovery from 6-day-long PEG	100	74.82 ab	67.78 ab	60.47 cd	49.01 bc
Janus	3 days on PEG	100	68.79 bc	0 a	0 a	0 a
	6 days on PEG	100	43.17 c	11.91 a	0 a	0 a
	3 days after recovery from 3-day-long PEG	100	73.03 ab	45.25 b	31.67 a	24.65 ab
	3 days after recovery from 6-day-long PEG	100	92.87 d	73.24 b	67.53 c	59.16 b
	6 days after recovery from 3-day-long PEG	100	66.04 c	45.60 de	28.07 d	11.12 a
	6 days after recovery from 6-day-long PEG	100	75.80 ab	79.31 c	63.44 cd	37.85 a
M49060	3 days on PEG	100	72.43 bc	2.75 b	0 a	0 a
	6 days on PEG	100	35.14 b	67.19 b	0.27 a	0 a
	3 days after recovery from 3-day-long PEG	100	71.35 ab	58.81 c	33.31 a	27.55 bc
	3 days after recovery from 6-day-long PEG	100	80.84 c	67.23 ab	59.87 ab	56.72 b
	6 days after recovery from 3-day-long PEG	100	50.41 b	43.03 cd	22.68 bc	11.07 a
	6 days after recovery from 6-day-long PEG	100	81.55 ab	76.42 c	66.00 d	46.07 b
2107	3 days on PEG	100	102.06 d	0 a	0 a	0 a
	6 days on PEG	100	42.84 c	5.46 a	0 a	0 a
	3 days after recovery from 3-day-long PEG	100	79.91 b	57.38 c	38.87 b	33.04 d
	3 days after recovery from 6-day-long PEG	100	99.75 d	86.38 c	75.85 d	71.67 c
	6 days after recovery from 3-day-long PEG	100	78.35 d	48.77 e	33.60 e	18.55 c
	6 days after recovery from 6-day-long PEG	100	81.95 b	74.64 bc	67.44 d	54.46 c

*: Letters in columns mean the differences between the genotypes in each treatment ($p < 0.05$).

bleach) for 1 minute and washed three-times with tap water and the germination test was started. They were germinated on filter paper moisten by 0, 5, 10, 15 and 20% PEG 600 solution in the dark at 24 °C. In 3 or 6 days, filter paper was changed with another that was moistening by water instead of PEG solution and germination was continued for further 6 days. The scheme of treatments is presented in Figure 1. Ten treatments from the combination of different concentration of PEG-solutions and from the duration of PEG treatments (3 or 6 days) were created and 60 seedlings were observed from every treatment in every observation time.

Observations and measurements were performed on 3rd, 6th, 9th and 12th days after starting the experiments (Figure 1). The length (SL, RL) and fresh weight (SW, RW) of shoots and roots on seedlings were measured, SL/RL and SW/RW quotients were counted and analysed statistically by ANOVA followed by Tukey's test. Before statistical analysis data were transformed and expressed in the percent of the control treatment in order to be able to make comparison between genotypes. Statistical analyses were performed using a computer program SPSS 9.0 for Windows. Experiments were repeated three-times.

Results and Discussion

Multivariate analysis of variance showed significant effects of osmotic stress treatments (=PEG treatments) and that of genotypes on the majority of morpho-physiological parameters measured and counted, except in the case of root weight concerning genotype effect, and also the genotype * treatment interaction was significant at $P < 0.05$.

The decrease both in the RL, RW and in the SL, SW was greater at 10%, 15% and 20% PEG treatment on the 3rd day than on the 6th day. The rate of decrease in the root parameters (RL, RW) was lesser than that of shoot parameters (SL, SW) in both observation times indicating that root growth was less affected by osmotic stress than shoot growth, similarly to the finding of others (Gadallah, 1996, Mullet & Whitsitt, 1996, Dobránszki et al., 2003) in other plant species, such as *Carthamus* or potato. Values of parameters measured at higher PEG concentrations (15%, 20%) were not significantly different in the average of 8 genotypes.

Relative growth of shoot and root subjected to osmotic stress and after recovery from osmotic stress in 8 pea genotypes are presented in Tables 1–4, where also the significant differences between the genotypes in each treatment are marked.

Significant differences in the shoot growth among genotypes were detected only at 5% PEG-treatment. Three main groups could be distinguished; SL and SW was the lowest in 'Baccara' and 'Erbi' and they were the highest in '2107', however, SL and SW did not differ significantly from its shoot parameters in 'M49060' and in 'Hunor'. The responses of genotypes to osmotic stress (=PEG-treatment) did not depend on the period of stress (3 or 6 days).

The variability in the root growth among genotypes was

the greatest at 15% PEG-treatment both in the case of 3-day-long and 6-day-long PEG-treatment. Three groups could be distinguished according to the statistical analysis: (i): 'Graphis', 'Erbi', 'Hanka', 'Baccara'; transitional cases: '2107' and 'M49060', (ii): 'Janus', (iii): 'Hunor'.

The other feature, which could be use for characterize the drought tolerance of a genotype, includes its growing response after cessation of water stress. Therefore, we have examined the shoot and root growth with 3 and 6 days after recovery from PEG-treatment searching differences principally among the genotypes. Studying the shoot growth, the greatest differences between the genotypes could be proved significantly with 6 days after recovery from 3-day-long 5% PEG-treatment. The main groups could be statistically distinguished are the followings in the case of SL: (i): 'Baccara'; 'M49060', '2107' (transitional to (ii)), (ii): 'Erbi', 'Hanka' (transitional to (iii)), (iii): 'Hunor', 'Janus' and (iv): 'Graphis' and that of SW: (i): 'Hunor', (ii): 'Graphis', (iii): 'Baccara'; 'M49060', 'Erbi', 'Hanka' and (iv): 'Janus', '2107'.

Considering root parameters the highest differences among the studied genotypes could be detected at 15 (and 20%) PEG-treatments with 6 days after recovery from 3-day-long osmotic treatment. The shortest roots were measured in '2107' and 'Hunor' and the longest ones in 'Hanka'. The RW was the lowest in '2107', 'M49060', 'Erbi', 'Baccara' and the highest in 'Graphis' and 'Hanka'.

With 6 days after recovery from 3-day-long 15% PEG-treatment the rate of SW to RW was significantly the highest in '2107', 'M49060' and the lowest in 'Hunor'. However, concerning the rate of SL to RL the tendency was reverse. Summarizing the results, in the case of '2107' and 'M49060' the low RW and high SW are characteristic with relatively long roots after cessation of stress treatments. However, their SW's were high but their SL's were relatively low. These *in vitro* responses may be associated with their relatively high drought tolerance of these genotypes in field.

Results from these experiments suggest that the germination characters and the early shoot and root growing ability after cessation of stress treatments could be more revealing for studying drought tolerance of pea genotypes, than their responses during stress conditions. Studying recovery of growing ability of pea seedlings after *in vitro* stress conditions can constitute a part of rapid selection of breeding material.

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References

- Dobránszki, J., Magyar-Tábori, K. & Takács-Hudák, Á. (2003): Growth and Developmental Responses of Potato to Osmotic Stress under *In Vitro* Conditions. *Acta Biol. Hung.* 54(3–4): 365–372.

Gadallah, M.A.A. (1996): Abscisic acid, temperature and salinity interaction on growth and some mineral elements in *Carthamus* plants. *Plant Growth Regul.* 20: 225–236.

Kpoghomou, B.K., Sapra, V.T. & Beyl, C.A. (1990): Screening for Drought Tolerance: Soybean Germination and its Relationship to Seedling Responses. *J. Agron. Crop Sci.* 164:153–159.

Levitt, J. (1980): Responses of Plants to Environmental Stresses. Vol.1. Academic Press, New York.

Lichtenthaler, H.K. (1996): Vegetation stress: an introduction to the stress concept in plants. *J. Plant Physiol.* 148:4–14.

Morgan, J.M. (1984): Osmoregulation and water stress in higher plants. *Ann. Re. Plant Physiol.* 35:299–319.

Mullet, J.E. & Whitsitt, M.S. (1996): Plant cellular responses to water deficit. *Plant Growth Regul.* 20: 119–124.

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

Sánchez, F.J., Manzanares, M., deAndrés, E.F., Tenorio, J.L. & Ayerbe, L. (1998): Turgor maintenance, osmotic adjustment and soluble sugar and praline accumulation in 49 pea cultivars in response to water stress. *Field Crops Research.* 59:225–235.

Sánchez, F.J., deAndrés, E.F., Tenorio, J.L. & Ayerbe, L. (2004): Growth of epicotyls, turgor maintenance and osmotic adjustment in pea plants (*Pisum sativum* L.) subjected to water stress. *Field Crops Research.* 86:81–90.