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EFFECT OF DROUGHT ON THE PERFORMANCE OF THREETURF GRASS SPECIES

ABSTRACT

Drought is the main environmental factor hampering world agriculture production. In the face of warming climate and reduced fresh water resources it become obvious that search for any factors decreasing water use is strongly recommended. Turf grasses able to withstand drought period longer could be recommended for turf areas as parks, lawns, home gardens etc. and relatively lower amounts of water should ensure satisfactory turf quality. Therefore, twelve turf varieties from three major cool-season turf grass species: perennial ryegrass (*Lolium perenne* L.), Kentucky bluegrass (*Poa pratensis* L.) and red fescue (*Festuca rubra* L.) were tested in glasshouse pot experiment and in the laboratory for determination of their relative ability to withstand green longer in the face of water deficit.

The different response of the examined forms to drought was noted. Conditions that favor fast water depletion were the most suitable for the expression of water deficit-related traits. Therefore, sandy mixture of 16% volumetric moisture content at field water capacity was mostly suitable for observation of the variation of tested forms. Turf condition of Kentucky bluegrass, as contrary to red fescue, was strongly connected with the soil moisture. Different manifestation of drought resistance was observed in tested species. Kentucky bluegrass, as rather no resistant to drought, exposed low level of drought avoidance. Red fescue was able to survive drought mainly due to leaf blades resistant to desiccation. In perennial ryegrass some other mechanisms evolved to survive drought. Early leaf wilting and senescence contributes to nutrient remobilization during drought and avoids large water loses during the transpiration. Therefore, perennial ryegrass turf was able to regenerate better after drought, as compared to the other tested grass species.

Search for new turf forms should focus on searching for ability to maintain acceptable conditions longer in a presence of increasing water deficit. It will then reduce the duration of period of poor turf conditions and further, turf water demands.

Key words: drought, grasses, turf, cell membrane stability, *Lolium perenne, Festuca rubra, Poa pratensis.*

Communicated by Dariusz Mańkowski

INTRODUCTION.

Drought, a period of abnormally dry weather, results in the soil-water deficit and subsequently – plant-water deficit. Arising from the moisture deficit in the soil and air, the drought interfere in the water balance of a given area (Bąk, Łabędzki, 2002). Water deficit in plant disrupts many cellular and whole plant functions, having a negative impact on the plant growth and reproduction. Crop yields are reduced by 69% on average when plants are exposed to unfavorable conditions in the field (Bray, 2001). In 2018 drought has affected a third of Poland's crop. Farmers across northern and central Europe are currently facing crop failure and bankruptcy as a consequence of the most intense regional droughts in recent memory (Nelsen, 2018).

In nature, certain species are adapted to the water deficits, exposing some resistance mechanisms and strategies. In case of grasses drought resistance is the ability to produce the desired product (herbage or green lawn) during slight, temporary or early drought; survive severe drought; and finally, having survived, respond rapidly to renewed water supply (Humphreys, 2001).

Cool-season turf grasses often suffer from extended periods of drought during the summer but water supplies used to irrigate turf are limited and are in competition for use by agriculture, recreation etc. It is in the best interest of turf managers to conserve water and to design both irrigation and turf which provide the quality of the grass with minimum water use (Hull, 1997). One of the frequently stated *turf disadvantages* is that it needs lots of water. But this is only a public perception, not scientifically accepted truth. High water use from turf areas is mostly related to human decision to irrigate the grass, not to the grass itself. Moreover, without a drop of water during drought periods, most of the currently used turf grass species survive (Żurek, 2006).

Although irrigation may be costly, a green and growing turf improves environmental conditions better than a brown turf. The main benefits of a healthy turf are water and wind erosion control. Actively growing turf may have a surface temperature that is 20 degrees cooler than a dormant turf during the summer (Hull, 1997).

Drought stress affects the turf quality, growth rate, evapotranspiration and recuperative potential (Ebdon and Kopp, 2004). Turf quality decline is an effect of reduction of root growth, leaf water potential, cell membrane stability, photosynthetic rate, photochemical efficiency and carbohydrate accumulation (Carrow, 1996 a, b; Huang and Gao, 1999; Jiang and Huang, 2001 a, b).

One from many important factors that determine the drought resistance of plants is the ability of leaf cell membranes to keep its function and integrity as long as possible during water deficit stress (Huang *et al.* 1997c). Membranes play a central role in various cellular functions, in particular those membranes with embedded enzymes and water/ion transporters. Drought leads to severe membrane damage. Efflux of water from the cells results in shrinkage of cell walls and plasma membrane, and eventually to

collapse of cells (Svensson, 2001). Therefore, the strain on membranes is one of the most important effects of drought and survival (Chaves and Oliveira, 2004). It is also closely linked with the plant recovery after drought, which is a function of plant capacity to avoid or to repair membrane damage. Grass dehydration tolerance is affected by cell membrane stability. On the basis of cell membrane stability selection for drought resistant turf grasses was suggested (Zhao *et al.*, 1994). In some experiments Kentucky bluegrass (*Poa pratensis* L.) ecotypes of higher cell membrane stability were also resistant to simulated drought conditions (Abraham *et al.*, 2003, 2004; Wang and Huang, 2004).

Numbers of experiments were described to examine different aspects of the turf grass performance during drought. Generally, turf performance, which is a complex feature combined from the sward density during growing season, turf color, texture etc. declines as drought stress increase. However, the range of decline and final regrowth after drought are strongly dependent on numerous factors as for example: genetic properties of plant, plant age, soil type, management intensity, drought duration etc.

Pot experiments in glasshouse or controlled environment are quite suitable and widely used. Despite of some disadvantages due to the age of plants (too young), unreal high transpiration rate and unrealistic fast root growth, it is still very convenient tool to examine the stress resistance of plants (Humphreys, 2001). Different pot dimensions and treatments were presented in numerous works, however various soil conditions in pots were not discussed intensively, including sandy soil structures usually found on well projected football pitches.

The aim of above work was to analyze the effect of different soil conditions, including general turf performance during a simulated drought with relation to the leaf cell membrane stability of three major turf grass species.

MATERIALS AND METHODS.

Twelve turf grass varieties and breeding lines (further referred to as objects) of major European turf grass species were selected for above experiment: perennial ryegrass (*Lolium perenne* L.): Stadion, Stoper, Nira and breeding strain - KRH-22, Kentucky bluegrass (*Poa pratensis* L.): Alicja, Ani and breeding strains: Dresa and Chałupy, red fescue (*Festuca rubra* L.): Adio, Bargena, Nimba and Leo. Seed was kindly provided by breeders or breeding companies. Three different soil mixtures were prepared from peat, river sand and compost soil as follows: ‘peaty’ mixture - 1 part of compost soil and 2 parts of peat; ‘proportional’ mixture – 1 part of compost soil, 1 part of sand and 1 part of peat; ‘sandy’ mixture - 1 part of compost soil, 1 part of peat and 4 parts of sand. Soil mixtures were than analyzed for the chemical, structural and water properties (Table 1).

Table 1

Chemical, physical and water properties of soil mixtures

Soil parameter:	Type of soil mixture:		
	Peaty	Proportional	Sandy
Chemical analysis results (in mg per 100g of soil)			
pH	7.3	7.6	7.7
Na	7.7	3.8	3.2
K	45.6	24.2	8.0
Ca	488.1	287.5	226.1
Mg	27.1	11.1	5.6
P	5.9	4.7	3.1
Salinity [g/100 g of soil]	0.17	0.07	0.05
$C_{organic}$ [g/1 kg of soil]	95.9	32.0	8.8
N_{total} [g/1 kg of soil]	5.5	2.8	0.6
C / N	18 : 1	11 : 1	15 : 1
Soil texture (share in %)			
< 0.02 mm	4	15.2	0.5
0.02 – 0.05 mm	6.3	22.4	6.2
0.05 – 0.1 mm	15.4	14.2	15.9
0.1 – 0.25 mm	16.5	12.2	34.1
0.25 – 0.5 mm	35.7	30.4	31.9
0.5 – 1.0 mm	22.1	5.6	11.4
Bulk density of soil [g×cm^3]	0.715	1.193	1.477
Water properties (in % of volumetric moisture content)			
Full water capacity (pf = 0.0)	70.7	52.4	42.8
Field water capacity (pf = 2.0)	47.7	31.4	16.4
Permanent wilting point (pf = 4.2)	13.9	9.0	6.2

Test procedures:

Pot experiment

Metal pots (6 per one object, 5000 cm^3, 20 cm in diameter, with drainage hole in the bottom) were filled with soil mixtures: 3.6 kg of peaty mixture, 6.0 kg of proportional mixture and 7.4 kg of sandy mixture per pot. On the basis of standard germination test results, sowing quantities were calculated to equalize seedling amount per pot area. Sowing quantities (in grams per pot) were as follows: Kentucky bluegrass: Dresa – 0.52, Chałupy – 0.54, Ani – 0.55, Alicja – 0.60; red fescue – Adio – 0.64, Leo – 0.67, Bargena and Nimba – 0.70; perennial ryegrass: Stadion – 0.94, Stoper – 0.96, Nira – 0.96 and KRH-22 – 1.01.

After sowing, pots were covered with 0,5 cm of the sand, well-watered and placed in the field. From seed sowing until seedling emergence pots were covered with white polypropylene non-woven cover and watered daily. During further vegetation in the field, pots were watered 3 – 4 times a week and grass was cut 3 times with hand mower at height of 3 cm. Mineral fertilizer was added once: 1.06 g per pot, and it equals (in kg per 1 ha): 30.9 kg N, 16.7 kg P, 49.6 kg K and 5.3 kg Mg.

Mean air temperature during grass vegetation in field (April, May and June) was 13.3°C and total rainfall – 149 mm.

After 80 days in the field, pots were moved to the unheated glasshouse, well-watered, weighed (W_1) and soil volumetric moisture content (VMC) was measured with *ThetaProbe* (*ThetaMeter* HH1, manufactured by Eijkelkamp Agrisearch Equipment, The Netherlands) at depth 0 – 6 cm.

Three pots per object in each of three soil mixtures were further kept without watering (drought conditions) and next three pots in each soil mixture were watered once a week with 0.5 l of tap water per pot (control conditions). VMC was measured two times per week and following traits were also evaluated:

- sward density (SD) was evaluated at the beginning and at the end of test, using 1-9 scale, where 1 is bare ground, no plants; 9 is complete turf cover (Prończuk, 1993; Prończuk *et al.* 1997),
- turf condition (TC), in 1 – 9 scale: 1 – completely dead plants, no green tissue visible, even when tillers dissected, 3 – trace of green tissue, usually at base of the youngest leaves, 5 – approx. half of plants with appreciable amounts of green leaves, 7 – most or all of leaves alive, but with the most of them scorched, permanently wilted, 9 – all leaves alive without symptoms of scorching (Humphreys and Thomas 1993, Minner and Butler 1985).

During vegetation in glasshouse pots were cut 6 times at 3 cm with hand mower. End of the drying phase was noted when VMC dropped to 0% and TC to 1. Regeneration started after pots weighed for the second time (W_2), and submerged in water for initial weight (W_1) recovery (ca. 24 hours). Further treatment for the test pots was the same as for control pots. VMC and TC were further measured and observed during regeneration. At the end of experiment (81 days after pots replacement to glasshouse, 161 days after sowing) SD was evaluated.

The total amount of available water for the test turf grown in pots was estimated on the basis of pots weights before and after drought ($W_1 – W_2$).

Mean air temperature during glasshouse test was 20.6°C, with optimum between fourth and sixth week (from 20.0 to 30.3°C, mean 24.9°C). Humidity ranges from 55.4 to 98.5%, with mean value at 75.7%. The highest humidity values were noted at the end of the test and lowest values – between eight and eleventh week.

Membrane stability

Cell membrane stability (CMS) was assessed according to Amin and Thomas (1996). Samples of ca. 80 mg of fully emerged, healthy and undamaged leaf lamina were collected from each tested object from control conditions grown in proportional mixture. Leaf samples were further incubated over silica gel for 24 h (drought simulation) or over water in humid chamber at 25°C for 30 minutes (control). Samples were than leached in 150 cm^3 of deionized water for 24 h on the laboratory shaker, and electrical conductance was measured (C_1). Samples were than autoclaved for 40 minutes, allowed to cool, and conductance was measured again (C_2). Membrane stability was calculated both for dried and control samples as :

$$CMS = 100 \times \frac{1 - C_1}{C_2}$$

CMS of dried leaves was expressed as a percentage of control samples.

Statistical analysis

All statistical calculations were made with STATISTCA ver. 12.0 PL. Significance of differences were accepted with 95% of probability. Lest significant differences (LSD) were calculated according to Fisher test and values were shown only if statistically significant with accepted probability.

For visual presentation of relations between VMC and TC, the best fitted equation were plotted, using the highest value of coefficient of determiantion (R^2). For all species and mixture types rule as:

$$y = a \times ln(x) + b$$

was used, where dependent variable is VMC and independent variable – TC).

RESULTS

Soil VMC changes during drying.

As it has been proved by regression analysis, soil moisture decrease during drying was linear in the case of all species and all used soil mixtures (Table 2). It is also evident from above that mean VMC of soil under sward dropped to 0% after 22 – 24 days in sandy mixture, from 28 to 29 days in proportional mixture and from 44 to 46 days in peaty mixture of drying. In each of applied soil mixtures, perennial ryegrass was the fastest soil-drying species, as contrary to Kentucky bluegrass.

Table 2

Results of the linear regression analysis with VMC as dependent variable and number of days since water withheld (drying) as independent variable.

Soil mixture	Species	Model	Unstandardized coefficients		Standardized coefficients	t	Sign.	Dependent variable value
			B	Std.Error	Beta			For independent = 0 (VMC = 0%)
Peaty	*Festuca rubra*	Constant	41.04	1.38		29.70	0.000	45.0
		No. of days	-0.91	0.05	-0.983	-19.90	0.000	
	Lolium perenne	Constant	40.25	1.60		25.14	0.000	44.3
		No. of days	-0.91	0.05	-0.977	-17.10	0.000	
	Poa pratensis	Constant	41.86	1.36		30.78	0.000	45.8
		No. of days	-0.91	0.05	-0.983	-20.24	0.000	
Proportional	*Festuca rubra*	Constant	26.96	0.89		30.37	0.000	28.9
		No. of days	-0.93	0.05	-0.989	-18.56	0.000	
	Lolium perenne	Constant	25.57	1.02		24.99	0.000	28.1
		No. of days	-0.91	0.06	-0.984	-15.76	0.000	
	Poa pratensis	Constant	29.72	1.12		26.60	0.000	29.5
		No. of days	-1.01	0.06	-0.985	-15.97	0.000	
Sandy	*Festuca rubra*	Constant	17.04	0.90		18.857	0.000	23.7
		No. of days	-0.72	0.07	-0.975	-10.677	0.000	
	Lolium perenne	Constant	16.14	0.78		20.604	0.000	22.1
		No. of days	-0.73	0.06	-0.981	-12.514	0.000	
	Poa pratensis	Constant	18.00	0.55		32.567	0.000	23.9
		No. of days	-0.75	0.04	-0.991	-18.233	0.000	

No statistical differences between grass species and objects were found for the amount of water available for plants during drying. It ranges from 2.217 kg H_2O per pot for peaty mixture, 1.355 kg H_2O per pot for proportional mixture to 0.915 kg H_2O per pot for sandy mixture.

Turf performance

As soil VMC declined from the field water capacity (pF = 2.0) to permanent wilting point (PWP, pF = 4.2), condition of turf (TC) was rather stable. First visible symptoms of the permanent water deficit on the turf was leaf wilting i.e. TC decrease to score 7. It includes a blue-green color and leaf rolling or folding (Carrow, 1996). In our experiment, perennial ryegrass varieties wilted first, in sandy mixture some varieties wilted half a day after VMC dropped to PWP (Table 3). As contrary to above, the longest delay of wilting was noted for Kentucky bluegrass 'Dresa' in peaty and proportional mixtures. Generally, if VMC reached PWP, turf become wilted after 3.3 – 5.4 days in peaty and proportional mixture but 0.4 – 4.1 days in a sandy mixture. As soil VMC decreased below PWP, TC also decreased and finally dropped to 1, after 27 days in sandy mixture, 35 days in proportional mixture and

53 days in a peaty mixture. Only in sandy mixture intra-specific variation was noted for perennial ryegrass and red fescue.

Table 3

Number of days from the beginning of drying to: decrease of soil moisture content to the permanent wilting point (pF=4.2); wilting of turf (TC=7) and its total dry-out (TC=1)

Name of object	Soil mixtures:								
	Peaty			Proportional			Sandy		
	pF=4.2	Wilting	Dry-out	pF=4.2	Wilting	Dry-out	pF=4.2	Wilting	Dry-out
	Mean for								
P. pratensis	29.6	35.0	54.1	18.6	24.0	35.3	13.7	17.8	28.9
Alicja	29.7	35.7	54.0	18.9	24.3	34.8	14.2	20.0	29.1
Ani	30.6	36.0	54.6	19.2	24.0	36.6	15.3	20.7	29.2
Chałupy	30.4	33.7	55.5	19.0	23.5	35.5	12.8	14.7	30.1
Dresa	27.5	34.7	52.4	17.4	24.0	34.4	12.5	16.0	27.2
	Mean for								
L. perenne	26.9	30.3	51.2	16.9	20.2	33.4	12.4	12.8	24.2
KRH-22	27.9	32.7	54.1	16.5	18.7	33.8	12.4	13.5	25.8
Nira	27.5	26.3	49.4	16.6	18.7	33.9	14.1	13.5	26.2
Stadion	27.0	32.3	52.3	17.2	20.3	33.5	11.6	12.0	23.2
Stoper	25.2	29.7	49.1	17.2	23.0	32.4	11.5	12.0	21.6
	Mean for								
F. rubra	28.1	31.8	54.2	18.6	23.6	35.7	13.0	16.3	28.2
Adio	27.4	26.3	56.8	18.1	23.0	34.0	9.8	13.0	24.1
Bargena	28.4	33.0	55.5	19.1	24.0	35.7	12.9	17.0	28.5
Leo	27.3	32.0	54.4	18.8	22.3	37.2	13.8	16.0	29.3
Nimba	29.1	35.7	50.0	18.3	25.0	35.7	15.4	19.0	31.0
Mean for objects:	28.2	32.3	53.2	18.0	22.6	34.8	13.0	15.6	27.1
LSD for objects	-	-	-	-	3.6 **	-	3.46 **	6.1 **	5.3 **
LSD for species	2.2**	3.8***	-	1.25***	1.8***	-	-	3.17*	2.8 **

Levels of significance: ** p < 0.05. *** - p < 0.01

Moisture decrease was not related to tested objects, however TC decrease to wilting (7.0) and total leaf dry out (1.0) was not only related to the soil mixtures but also to tested objects (Table 4).

Table 4

Two-way ANOVA results for moisture and condition decrease (mean squares).

Source of variation	VMC decrease to permanent wilting point	Decrease of turf condition to:	
		7.0	1.0
Soil mixtures	2038.0 **	2451.0 *	5977.5 **
Objects	8.35	70.9 **	26.7 *
Soil mixtures x objects	4.2	12.6	10.1

Statistical significance at: * -p < 0.05; ** - p < 0.01

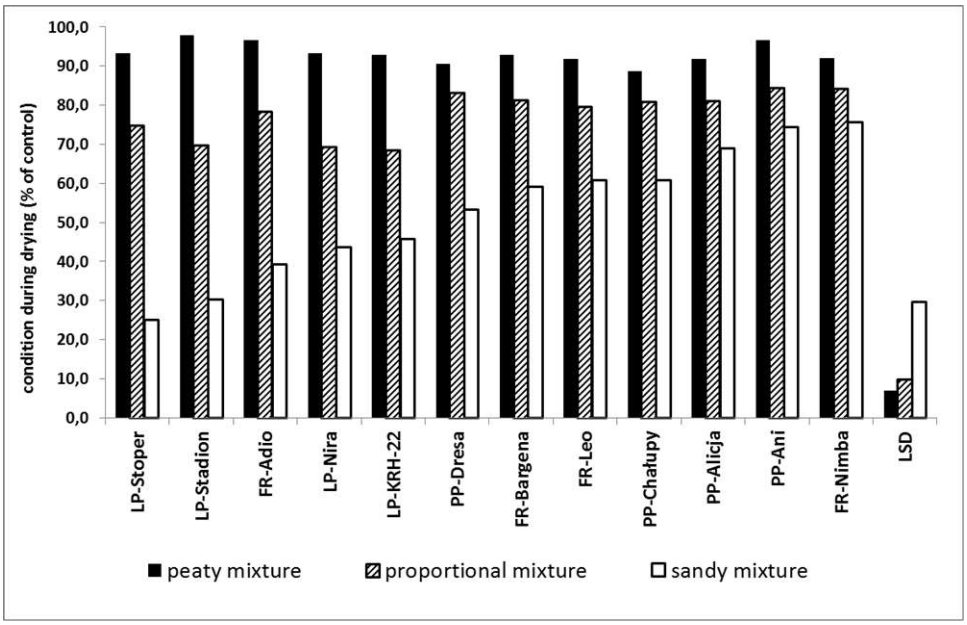

Fig. 1. Turf condition during drying (% of control pots, objects ordered with increasing values in a sandy mixture)

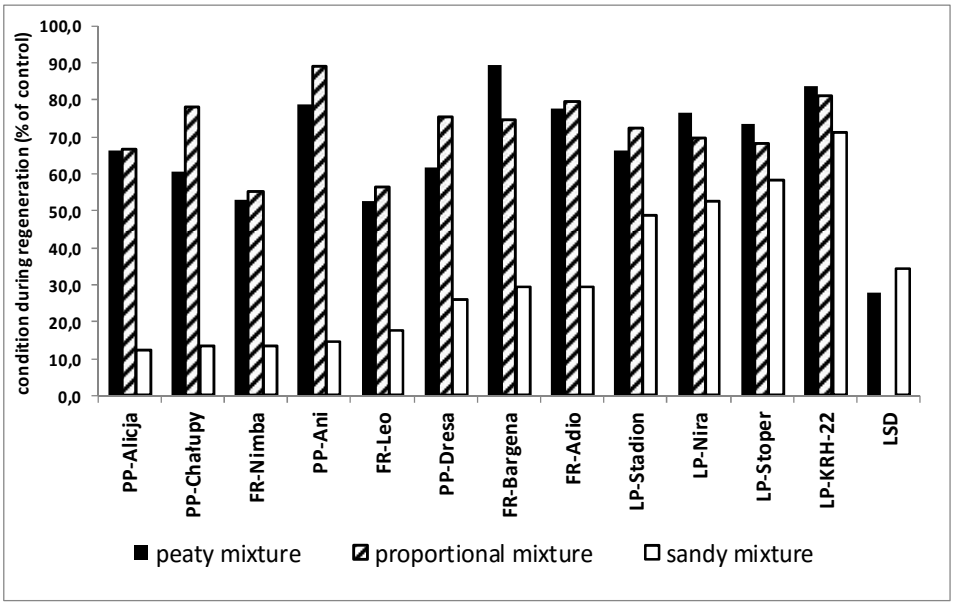

Fig. 2. Turf condition during regeneration (% of control pots, objects ordered with increasing values in sandy mixture). Abbreviations: LP – *Lolium perenne*. FR – *Festuca rubra*. PP – *Poa pratensis*

Mean values of TC during drying were strongly reduced in a sandy mixture, and difference between objects were also noted in a proportional mixture (Fig. 1).

The strongest effect of water deficit was noted in perennial ryegrass, where average TC was only 5.7% of control conditions for the grass grown in a peaty mixture, 29.4% in a proportional mixture and 63.9% in a sandy mixture. Condition of perennial ryegrass variety Stoper dropped to 25% of control in a sandy mixture, as compared to red fescue Nimba (75.6%) and Kentucky bluegrass Ani (74.4%).

Most of the tested objects were not able to regenerate to the values of control turf (Fig.2). In the case of Kentucky bluegrass and red fescue very low values of TC (from 1.1 to 2.4) were observed. It means that only few green plants per pot regenerated. As contrary to above, the quality of perennial ryegrass after regeneration was more than 50% of control. For example, perennial ryegrass KRH-22 regenerated in all soil mixtures to the level of control conditions. Kentucky bluegrass regenerated better in a proportional mixture, than in a peaty and sandy. For red fescue (exc. Bargena) there was no difference between regeneration in a peaty and proportional mixtures.

Reduction of SD was the highest for Kentucky bluegrass varieties grown in sandy mixture where it decreased to 25% (Chałupy) and 31% (Dresa) of its initial value. As contrary, the best SD after drought and regeneration was noted for perennial ryegrass: from 70% (in a sandy mixture) to 93% (in a peaty mixture) of initial density. For perennial ryegrass KRH-22 no statistical difference between all mixtures used was found (Fig. 3).

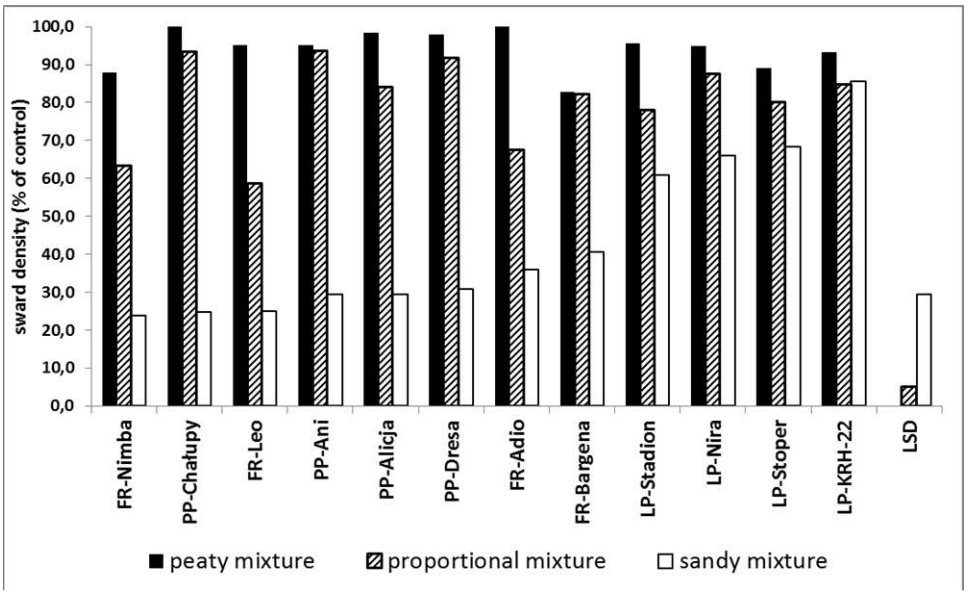

Fig. 3. Sward density at the end of test (% of initial value, objects ordered with increasing values in sandy mixture). Abbreviations: LP – *Lolium perenne*. FR – *Festuca rubra*. PP – *Poa pratensis*

Reduction of the sward density after drought and recovery in all soil mixtures was noted for red fescues. It ranged from 23.8% of initial SD in a sandy mixture (Nimba), 58.2% in a proportional (Leo) to 83.0% in a peaty mixture (Bargena). No SD reduction was noted for Adio in peaty mixture.

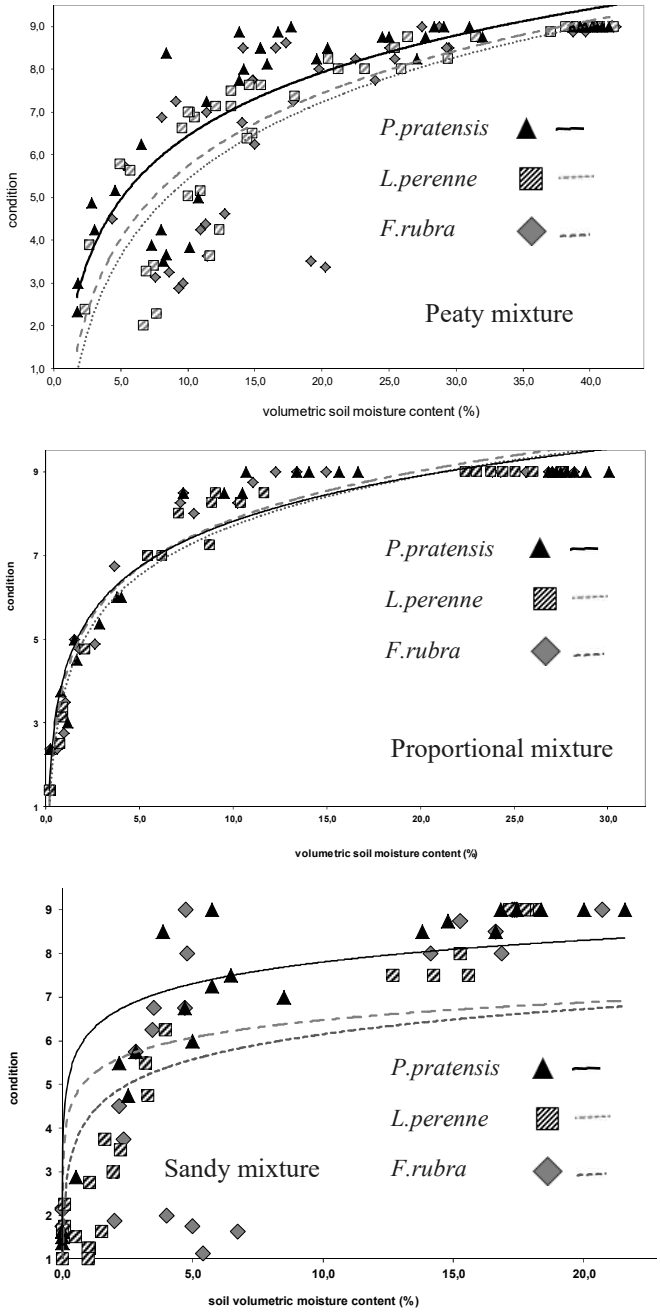

Fig. 4. Relation between VMC (volumetric soil moisture content) and TC (turf condition) in different soil mixtures

It is evident from above that turf condition during drying is close related to VMC of soil. When plotting all TC data from different soil mixtures against

VMC (Fig. 4) it can be concluded that condition of Kentucky bluegrass is mostly related to the soil moisture during drying and regeneration. Variation of TC is described by VMC from 74% in a peaty mixture to 92% in a proportional mixture. As contrary, TC of red fescue was the least related to VMC. Generally, TC during drying and regeneration was mostly dependent on VMC in a proportional mixture: from 92% for Kentucky bluegrass to 97% for perennial ryegrass.

Cell membrane stability (CMS)

Stability of the leaf cell membranes of tested objects was the highest for red fescue: from 45.5% for Nimba to 19.3% for Bargena (Fig. 5). No significant difference was noted for perennial ryegrass entries (CMS from 10.6 to 9.2%). Significantly lower CMS value were noted for strain Chałupy of Kentucky bluegrass (7.2%), while for the rest of objects from the same species it raged 17.2 (Alicja) to 13.5 (Dresa).

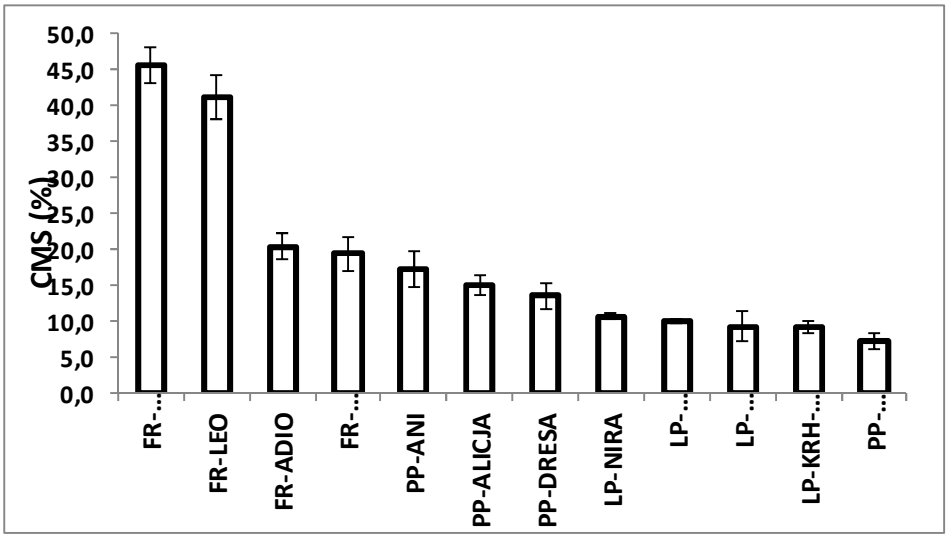

Fig. 5. Leaf cell membrane stability of tested objects – expressed as a % of control (non-dried leaves) LSD = 5.48, Abbreviations: LP – *Lolium perenne*. FR – *Festuca rubra*. PP – *Poa pratensis*

Relations between CMS and TC during drought were different for the tested species (Table 5). For Kentucky bluegrass SD after drought and number of days from water withheld to wilting were positively correlated with CMS. However, for red fescue and perennial ryegrass only few correlations were significant and all of them negative, mostly for the turf grown in sandy mixture. Above relation seems to be 'species-specific' and therefore general conclusion is not quite clear. We can only suppose that during fast drying in our experimental conditions higher membrane stability was not the main factor that determined post-drought performance for red fescue and perennial ryegrass

Table 5

Correlation coefficients for traits observed on turf and cell membrane stability (only statistically significant coefficients were given).

Traits	Soil mixture	*Poa pratensis*	*Lolium perenne*	*Festuca rubra*
Mean turf condition during drying (% of control pots)	Peaty	-	-	-
	Proportional	-	-	-
	Sandy	-	-	-
Mean turf condition during regeneration (% of control)	Peaty	-	-	-
	Proportional	-	-	-
	Sandy	-	-0.90*	-0.93*
Sward density after drought & regeneration (% of initial value)	Peaty	-	-	-
	Proportional	-	-	-
	Sandy	0.99*	-	-0.90*
Soil moisture decrease to permanent wilting point	Peaty	-	-	-
	Proportional	-	-	-
	Sandy	-	-	-
Number of days from water withheld to wilting start	Peaty	-	-	-
	Proportional	0.90[a]	-	-
	Sandy	-	-	-
Number of days from water withheld to total dry out	Peaty	-	-	-0.91*
	Proportional	-	-	-
	Sandy	-	-	-

Significance levels: a – $p < 0.1$; * - $p < 0.05$

DISCUSSION.

Water availability is one of the major factors that determine seed germination and further plant development in the regions of seasonal or permanent water deficits (Dodd and Donovan, 1999; Sharma, 1973). The rate of the water uptake is crucial for the plant competitive ability (Wilman *et al.*, 1998). Soil moisture changes observed in our work were quite similar to described by Jiang and Huang (2000) for Kentucky bluegrass and Volaire and Leliévre (2001) for the orchard grass and tall fescue. In our experiment we have noticed that soil moisture content in pots with perennial ryegrass decreased faster then in pots with Kentucky bluegrass or red fescue. Wilman and co-authors (1998) suggested that at spring, perennial ryegrass absorbed water slower than tall fescue or Italian ryegrass, but from May to October it was similar for all species, even with advantage to perennial ryegrass. Such relation is due to intensive growth of perennial ryegrass green mass, especially during the second part of growing season (Falkowski, 1982; Elberse and Berendse, 1993).

Rate of the soil moisture decrease to the level of permanent wilting point (in our experiment between 10 to 30 days of drying) was similar to results obtained in pot experiment made by Karsten and Mac Adam (2001) with perennial ryegrass and tall fescue. They noted permanent wilting point between 10 and 20 days of drying.

Soil moisture changes are strongly connected with the changes in plants. Plants cope with soil moisture decrease to level close to permanent wilting point. Below this level some reversible changes (e.g. desiccation of leaf bases) may first occur, and further, irreversible changes (e.g. total dry-out) will affect plant (Chaves *et al.*, 2002; 2003; Chaves and Oliveira, 2004). At the beginning of soil moisture decrease the plants are usually able to keep tissue water potential at unchanged level, but if drought still progresses, rapid decrease of tissue water potential appears (Amiard *et al.*, 2003).

First, reversible change associated with the water deficit is leaf wilting. Wilt symptoms include a blue-green color and leaf rolling or folding (Carrow, 1996a, b). When wilted, the plants may reduce transpiration and therefore, total water loss. According to Thomas and Evans (1989) wilting intensity is close connected with leaf water potential. It has been proved for Kentucky bluegrass by Ebdon and Koop (2004) that degree of wilting was connected both with the ability to extract water from deeper soil layers and higher evapotranspiration. Observations on tall fescue leaf folding during the water deficit were more suitable for the estimation of plant water status than laboratory measures of leaf osmotic potential (White *et al.*, 1992).

Concerning fast wilting of perennial ryegrass, as was noted in our experiment, it could be the element of plant strategy for copying with drought. Jones (1990, after Milnes *et al.*, 1998) claims that the optimum drought copying strategy of plants is based on fast uptake and spending of water, provided that plant is able to survive water deficit due to special mechanisms. Such reactions has been noted for junegrass or orchard grass from South of Europe (Volaire, 1995; Milnes *et al.*, 1998). Very fast wilting of perennial ryegrass during drought was also probably due to the low root weight and low root:shoot ratio, as compared to Kentucky bluegrass and red fescue (Dziamski *et al.* 2007). Differences in the root distribution during drought stress may be due to carbon relocation from the shoots to roots for formation of a more extensive root system into deep soil (Mc Cann and Bingru Huang, 2008).

The main factor that determined wilting speed was the amount of water available for plants. The phenomenon of fast wilting on the light, sandy soils as contrary to a peaty soils, was observed during spring drought 2000 in the upper Noteć valley (Łabędzki, 2000). Different speed of wilting and dry-out was observed also by Carrow (1996 a) for tall fescue turf varieties.

It is possible that wilting was assisted by leaf cell membrane stability (CMS). We have noticed close relation between CMS and wilting for red fescue and Kentucky bluegrass. Red fescue Nimba of the highest CMS (45.5%) wilted after 36 days of drought in a peaty mixture while red fescue Adio, of low CMS (20.3%) wilted 10 days earlier. Kentucky bluegrass Alicja (high CMS = 15%) wilted 2 days later than Kentucky bluegrass Chałupy, of the lowest CMS = 7.2%. Such relation is rather easy to explain and has been proved in some other experiments, where Kentucky bluegrass ecotypes of higher cell membrane stability were also resistant to simulated drought conditions (Abraham *et al.*, 2003, 2004; Wang and Huang, 2004).

Some authors suggested that cell membrane stability during dehydratation was a measure of dehydratation tolerance of whole plants (Huang *et al.*, 1997

b). It was true for spring wheat (Zagdańska and Pacanowska, 1979). However, according to our results, high cell membrane stability seems not to be the good measure of turf quality after drought. In perennial ryegrass we have noticed small variation of CMS while in red fescue rather high. The manifestation of CMS is probably due to the general genotype drought resistance strategy and is close related to the share of other parts of plant in general drought resistance.

Next visible step in turf grass reaction to the permanent water deficit was leaf yellowing and finally, senescence and total plant dry-out. Leaf yellowing is the result of chlorophyll degradation in senescing leaves, which unmasks the presence of carotenoids (Munne-Bosch and Alegre, 2004). Drought-induced leaf senescence contributes to the plant survival under drought, since it allows an early diversion of resources from vegetative to reproductive development, remobilization of nutrients from drying leaves to the young parts of plant (thus contributing to plant survival) and reduction in water loss from the whole plant (Munne-Bosch and Alegre, 2004; Volaire *et al.* 2005). Leaf yellowing and senescence is normally considered a measure of drought tolerance in the field conditions (Beard, 1989; Carrow, 1996a,b; Huang *et al.*, 1997 a; Minner and Butler, 1995).

Different leaf senescence and further regeneration are of the key role for breeding lines evaluation because of high level of differentiation between genotypes (Thomas, 1990; Carrow, 1996a). However, for Kentucky bluegrass there was no close relation between the water use efficiency and leaf wilting and senescence (Ebdon and Kopp, 2004).

Soil water abundance was crucial for the plant condition during drying. In a soil mixture of the poorest water retention (sandy mixture), the lowest condition was noted. The differentiation of both species and varieties increased along with the decrease of water retention of soil mixtures. The most drought susceptible species was perennial ryegrass. It was the consequence of fast soil drying, as it was mentioned above. However, if plant reduces its foliage fast, shorter is the period of exposition of living, aboveground parts of plants to drought conditions, and therefore, less is the damage of leaf bases and tillers. Plants may therefore regenerate faster after the drought is over (Munne-Bosch and Alegre, 2004). One of the major factors that determine plant survival during drought is the effective water use. Higher water use efficiency for perennial ryegrass as compared to tall fescue and orchard grass was observed in pot experiment made by Johnson and Basset (1991, after Thomas, 1994). It was true for drought test conditions as well as for watered control pots. Competitive ability of perennial ryegrass in comparison with orchard grass, meadow fescue or timothy decreased in the wet conditions while with increasing site moisture, sugars content in dry matter decreased (Falkowski *et al.*, 1986; Baryła and Warda, 1999).

When the drought ends, after natural rain or artificial watering, the regeneration begins. It is of the major importance for perennial grasses, especially regeneration from existing plants rather than requiring establishment of new plants (Kemp and Kluvenor, 1994). Good regeneration after drought may be more important than plant growth during the dry season. Above process is linked with the ability to reduce cell membrane damages during desiccation or fast repairing of membranes (Chavez and Olivera, 2004). Regeneration is also dependent on

the density of tillers surviving prolonged water deficit, its regrowth and growth of new tillers (Volaire *et al.*, 1998). Water content in young plant tissues of leaves and sheets increase along with increasing soil moisture content (Amiard *et al.*, 2003). During the same time, water content in mature leaf blades increased rather slow, and in roots no increase was noted.
In our experiment none of tested objects regenerated to 100% of initial value, as it was noted in the previous field experiment on turf grasses (Żurek, 2000). Apart from obvious differences between pot experiment and field drought, perennial ryegrass was selected as the best regenerating species in the most stressful conditions (i.e. sandy mixture). Perennial ryegrass varieties regenerated its condition to 59% of control pots, while red fescue only 22% and Kentucky bluegrass – 16%. Fast regrowth of perennial ryegrass tillers after drought was also observed by Volaire and co-authors (1998). It was ascribed to greater availability of carbohydrates and proline at the end of drying phase (Volaire *et al.*, 1998).

One from many important factors that determine the quality of turf varieties is sward density (Diesburg *et al.*, 1997). Value of sward density at the end of the drying period was claimed to be a turf quality index (Qian and Engelke, 1999). Once more, perennial ryegrass seems to be a species of the highest sward density after drought and regeneration. Sward density of ryegrass was 70% in a sandy mixture, while for red fescue – 31% and for Kentucky bluegrass – 29%. Low sward density of red fescue turf after drought was also observed by Minner and Butler (1985).

Red fescue indicate some evolutionary developed adaptive mechanisms similar to f.e. *Trichloris crinita*, Argentinian pasture grass (Greco and Cavagnaro, 2003). Along with the increasing temperature and increasing probability of summer drought occurrence, red fescue plants decrease aboveground weight together with an increase of root growth production (Dziamski *et al.*, 2007). That is probably the reason why condition of red fescue sward was the least dependent on the soil moisture, as compared to Kentucky bluegrass and perennial ryegrass. An increase of root:shoot ratio is an element of the plant drought resistance strategy known as *drought avoidance* (Beard 1989; Chaves *et al.*, 2003). However, usually it is not in line with turf user expectations. Creeping red fescue and sheep fescue are commonly used for low maintenance turf (Dernoeden *et al.*, 1994, 1998, Diesburg *et al.*, 1997, Harkot and Czarnecki, 1999, Lutyńska, 1993). However, along with increasing drought, turf from above species usually display a brown patchy appearance, rather than uniform dormancy as for the perennial ryegrass and Kentucky bluegrass. Mulch, provided by dead leaves or dormant turf is difficult to mow. Finally, the dead areas on turf of creeping red fescue or sheep fescue never fill in with new growth and that is why above species were recorded to be less drought resistant than perennial ryegrass and Kentucky bluegrass (Minner and Butler, 1985).

Drought tolerant cultivars of the major amenity grasses were always very important for breeders and managers. But it is still not clear that any drought tolerant cultivars exist, even in large turf trails in USA (Thorogood 2003). On the other hand it is quite easy to find significant differences between commercial cultivars, on the single trait basis (Żurek, unpublished data). What is proba-

bly of the major interest, is that drought tolerance is species-dependent. In few cases species with genetically enhanced drought tolerance were used for the improvement of other species. One good example is hybridization between Texas bluegrass (*Poa arachnifera* Torr.) and *Poa pratensis*. A few of the resulting cultivars demonstrated enhanced drought tolerance (Read and Anderson, 2003).

However, considering the world-wide water problems, probably the best way is to look for 'water-saving' cultivars rather than for better drought tolerance. Presently the modern cultivars with improved drought resistance retain proper turgor in stress conditions by more intense transpiration. Unfortunately, more intense transpiration during the drought period is compatible with higher transpiration rate under the optimal weather conditions, which results in larger amount of water evapotranspiration from the soil (Rybka and Żurek, 2010). Nothing is currently known about the possibility of producing 'water-saving' amenity grass cultivars. Another option for the water problems is to use municipal water, wastewater, storm water or other types of water not suitable for people or animals. The idea is to have more than one water source available for use on a single turf site (Duncan *et al.* 2009).

CONCLUSIONS

1. The expression of natural variation, connected with the water deficit is much more visible in conditions that favor faster water loss. Environmental conditions have much stronger effect on the water loss than morphological and physiological properties of grass plants. Soil mixture, which was the most suitable for estimation of the grass reaction to the water deficit was a sandy mixture, of relatively low water capacity (ca. 16% of field water capacity). While testing in such conditions, differences between tested varieties were clearly manifested.
2. Key traits in the evaluation of turf grass in drought conditions seems to be regeneration and sward density maintenance after drought. Other biochemical and physiological parameters (e.g. leaf cell membrane stability) of great value for the general understanding of plant reaction to the stress, should be treated as additional determinants of potential quality of tested varieties.
3. Suggestions for the practice resulting from above publication and concerning turf surfaces quality during prolonged water deficits are that we should use grass mixtures with high perennial ryegrass contents to ensure relatively high turf regeneration after drought if watering is not possible or not recommended. In case of no problems with watering turf quality could be increased with higher share of Kentucky bluegrass in the mixtures.

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THE INFLUENCE OF NEAR INFRARED STIMULATION ON THE GERMINATION ENERGY AND GERMINATION CAPACITY OF SELECTED PARSLEY VARIETIES

ABSTRACT

A variety of different physical methods are used increasingly frequently to improve the quality of the seeds material. Numerous publications confirm the positive effect of, for example, the magnetic field with a frequency of 50 Hz on the germination of seeds. However, there are no significant reports regarding the impact of NIR radiation on the growth and development of plants. To fill this gap the influence of three doses of near-infrared seeds stimulation was tested under laboratory conditions. The power density was 6.9mW/cm2. The used seeds encompassed four parsley varieties: Konika, Osborne, Alba and Hanácká. Both the influence of the used variety as well as the radiation dose on parsley germination were tested. The obtained results indicate that the stimulation affects the selected parsley varieties.

Key words: biostimulation, germination energy, near infrared (NIR), parsley, seeds

INTRODUCTION

The aim of this publication was to investigate the impact of the near infrared radiation (NIR) on parsley seeds and to its influence on the growth and development of this plant.

The main objective was to increase the germination capacity of parsley seeds. Root parsley is a staple flavouring plant grown in Poland. However, harvesting parsley crop is not easy due to several factors. First of all parsley seeds have low germination capacity. Besides, they have high requirements for germination places, the floor should be

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middle-concise, rich, not crusted over, mould, and also it should have high water capacity and pH close to neutral. Parsley is very sensitive to rainfall, both deficit and overflow of water have a negative impact on its growth. Another factor is soil fertilization which is also necessary for parsley to grow.

The need for quality food is becoming an enormous challenge for the modern world. The quality of products available on the market is plummeting, however, simultaneously their prices are rising. The reason for this is the massive use of chemicals in the production process. Although these chemical agents are highly effective, they also pose a biohazard, because they penetrate food (Anisimov and Chaikina, 2014; Grabowska *et al.*, 2014; Grémiaux *et al.*, 2016; Khan *et al.*, 2009; Michalak *et al.*, 2018; Vasilenko, 2016; Wiatrak *et al.*, 2016).

The above mentioned threats resulted in greater consumer awareness and emphasised the necessity to conduct research on improving the quality of food. These investigations were connected with a significant increase in the interest in the use of physical stimulus factors to improve the quality of the plants. Physical stimulation influences the physiological and biochemical changes of plant seeds. However, it is important that this method does not penetrate into the soil substrate, and therefore it is not harmful for the environment (Aladjadjiyan, 2012; Bae *et al.*, 2015; Godlewska *et al.*, 2016; Michalak *et al.*, 2015; Podleśny, 2004; Saberi and Tamian, 2014; Tadeusiewicz, 2008). In the literature there are also descriptions of experiments in which the influence of near-infrared radiation (NIR) on the germination energy and the germination capacity of seeds, including parsley, were investigated (Grabowska and Mech, 2015a, 2015b; Gruszecki, 2005).

In general, NIR radiation works at the quantum level (by affecting the atomic and molecular levels), however, it also affects the level of plant cells and tissues. It has been known for a long time that the use of near-infrared radiation improves seed germination (although the mechanism of this process is not fully understood yet) (Michalak *et al.*, 2018). The ritish patent GB 2.303.533 indicates the possibility of stimulating seeds by means of near-infrared radiation, also when it is connected to red light (Vasilenko, 2016). It was shown in the literature that usually the stimulation of seeds by radiation in the wavelength range from 800 to 1000 nm improved the germination of various garden plants (Grabowska *et al.*, 2015, 2014, 2013; Johnson *et al.*, 1996; Niemczyk, 2017; Vasilenko and Popova, 1996). Moreover, the germination capacity of the seedlings increased when NIR light was applied.

The examination of the influence of near-infrared radiation on the parsley seeds of the selected varieties was the goal of the experiment presented in this publication. For this purpose, seed irradiation was performed with a halogen lamp with an NIR filter, and then the seeds were transferred to a phytotron. Four parsley varieties – Konika, Osborne, Alba and Hanácká were used in the experiment. It can be said that the experiment had a bifactor nature. The influence of the used variety and also the dose of radiation on parsley germination was also analysed. First 100 seeds were plated on blotting paper in a Petri dish. Then, the Petri dishes with seeds were placed in the phytotron until germination started. The tests were performed

three times. The temperature and humidity in the phytotron remained at the same level. Subsequently, 10 days after sowingthe germination energy of all samples was checked and 21 days after sowing – germination ability. The obtained results indicate that stimulation affects the selected parsley varieties. During the research, tests were also carried out to determine the structure of parsley shoots after stimulation and their properties were compared with a control sample that was not stimulated. The results of these studies will be subject of another publication.

MATERIALS AND METHODS

Biological material

The experimental material were four varieties of parsley seeds: Konika, Osborne, Alba and Hanácká. Root parsley is one of the basic, flavouring crops, commonly grown in Poland. It has been known in Poland for a long time, but its crop is not always successful. Failures in the production are due to several factors (Gruszecki, 2005). The seeds used in the experiment came from "MoravoSeed" and "Nohel-Garden" companies (Fig.1A).

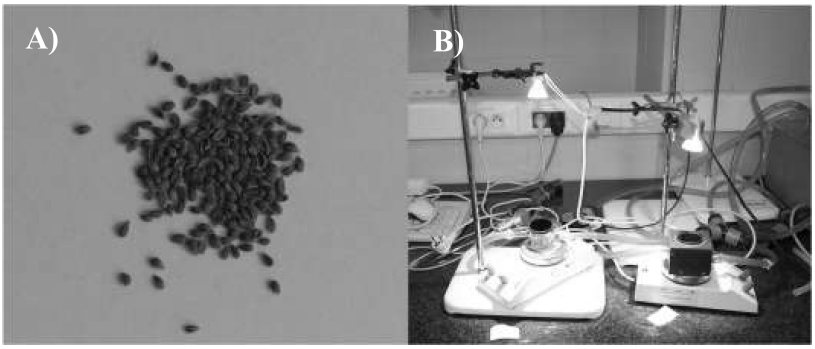

Fig. 1 A) Seeds of parsley B) Appliance for irradiation

Near-infrared procedure

The samples were exposed to halogen lamp radiation of a halogen lamp (Fig. 1B) equipped with a 700–2,000 nm filter. The light was focused on a flat glass tube in which the samples were kept in a dark glass cell. The power density of the incident light was 6.9 mW/cm2. It was measured by a fito-phytometer, OPTEL (Fig. 2). The irradiation temperature was kept constant at 21±1ºC by means of an additional water-cooling system (Walski *et al.*, 2014) (Fig. 3).

Research material consisted of four groups: without stimulation (control group), 15 min, 30 min and 60 min. When irradiation was switched off the samples were put into a phytotron for 20 min.

Fig. 2. Fito-photometer - device used to check power density

Fig. 3. Thermostat

Seeds storage

One hundred seeds were plated on blotting paper in the Petri dish (Fig. 4A). Then, the Petri dishes with seeds were located in the phytotron (Sanyo Versatile Environmental Test Chamber MCC-351H) until germination. Temperature and humidity were controlled throughout the entire process (Fig. 4B).

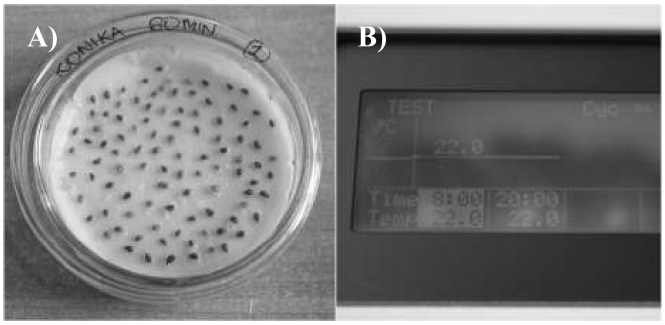

Fig. 4. A) Seeds plated in Petri dish, B) Temperature in phytotron

RESULTS AND DISCUSSION

The following parameters were calculated after stimulation: germination energy (10 days after sowing) and germination ability (21 days after sowing).

Germination energy (GE)

Germination energy is used to determine the viability of seeds. It is the percentage of the number of seeds germinated normally at the shortest possible time (Drozd *et al.*, 2004). On the 10th day after sowing, the germination energy of individual seed varieties was calculated for each of the applied radiation doses and also for each repetition (Table 1). Figures 5-8 show the seeds on selected Petri dishes 10 days after sowing.

Table 1

Germination energy of all extracted varieties of parsley seeds

Time [min]	KONIKA [%]			OSBORNE [%]			ALBA [%]			HANÁCKÁ [%]		
	1	2	3	1	2	3	1	2	3	1	2	3
0	82	82	78	92	84	90	63	55	70	56	59	50
15	83	73	72	86	86	80	64	41	68	52	60	69
30	75	74	85	85	90	89	64	66	58	58	61	64
60	75	79	93	85	79	2	58	64	70	55	51	69

Stimulation with near-infrared radiation does not affect the GE of Konika parsley seeds (Fig. 5), and in some cases even a slight decrease in germination energy can be observed. In the case of the Osborne variety (Fig. 6), near-infrared stimulation did not affect the GE of seeds. In the case of the germination energy evaluation for sample 3, which was stimulated for 60 minutes, a very low level of this parameter was observed due to the drying of the plate. The NIR stimulation did not affect the GE of Alba (Fig. 7) and Hanácká parsley seeds (Fig. 8), either.

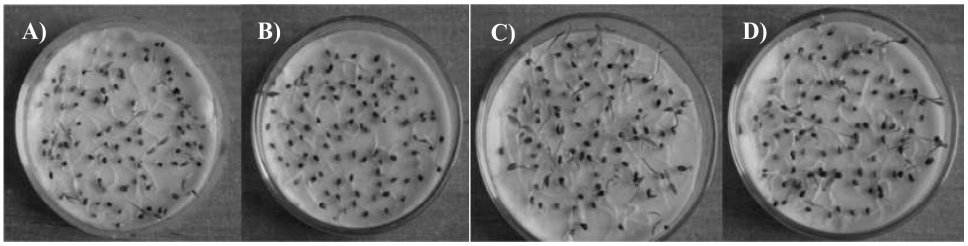

Fig. 5. Sample dishes with Konika seeds 10 days after sowing
A) Control sample, B) 15 min stimulation, C) 30 min stimulation, D) 60 min stimulation

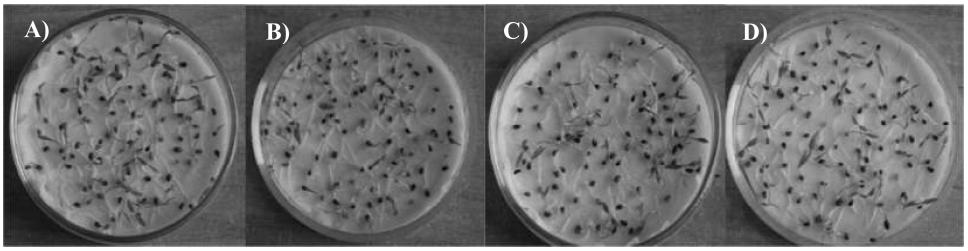

Fig. 6. Sample dishes with Osborne seeds 10 days after sowing
A) Control sample, B) 15 min stimulation, C) 30 min stimulation, D) 60 min stimulation

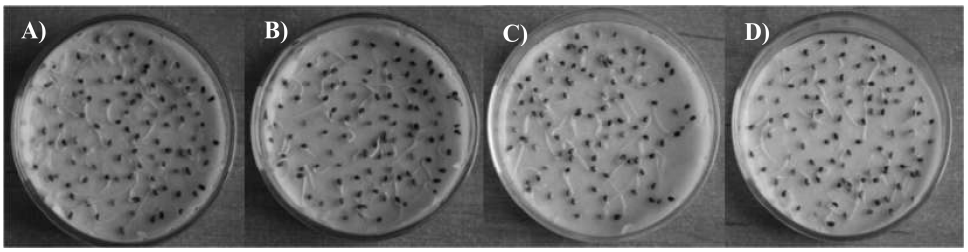

Fig. 7. Sample dishes with Alba seeds 10 days after sowing
A) Control sample, B) 15 min stimulation, C) 30 min stimulation, D) 60 min stimulation

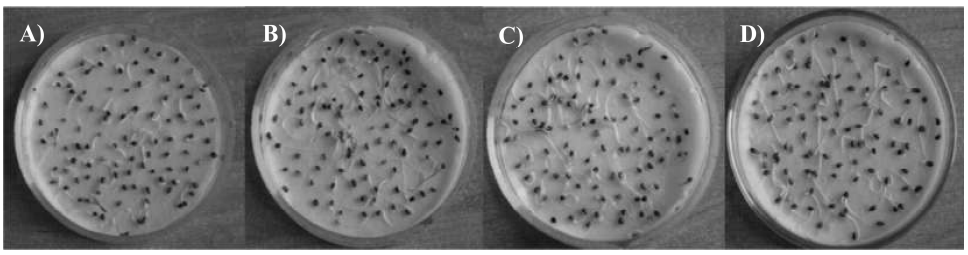

Fig. 8. Sample dishes with Hanácká seeds 10 day after sowing
A) Control sample, B) 15 min stimulation, C) 30 min stimulation, D) 60 min stimulation

Germination capacity (GC)

The term germination capacity is used to determine the percentage of seeds producing seedlings classified as normal under appropriate conditions in a defined period of time (Drozd *et al.*, 2004). Seeds stimulation by the near-infrared apparently influenced on the increase in germination capacity (Table 2) in seeds which were stimulated for 60min.

Table 2

Germination capacity of all extracted varieties of parsley seeds

Time [min]	KONIKA [%]			OSBORNE [%]			ALBA [%]			HANÁCKÁ [%]		
	1	2	3	1	2	3	1	2	3	1	2	3
0	83	84	82	94	98	90	77	68	74	66	70	58
15	85	81	75	89	89	81	72	82	79	62	73	72
30	78	82	88	92	90	94	82	88	76	63	61	77
60	85	86	95	86	83	84	81	83	85	64	72	75

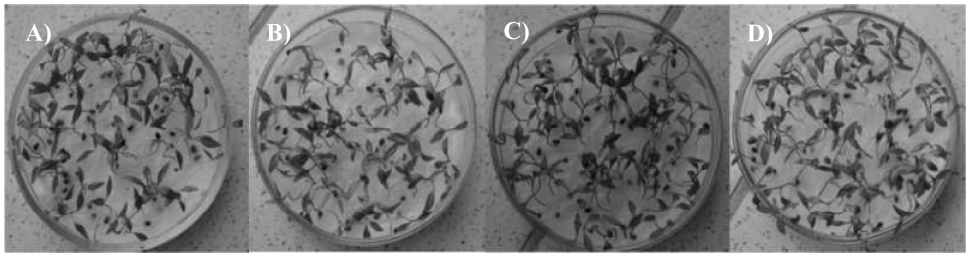

Fig. 9. Sample dishes with Konika seeds 21 days after sowing
A) Control sample, B) 15 min stimulation, C) 30 min stimulation, D) 60 min stimulation

After the stimulation of the Osborne parsley seeds stimulated 15 and 60min by near-infrared radiation, a decrease in the GC of these seeds becomes visible. NIR stimulation increased the GC of Alba parsley seeds, the biggest changes were observed in the case of 60min stimulation. Likewise, NIR stimulation increased the GC of Hanácká parsley seeds of varieties and again the biggest changes occurred in the case of 60min stimulation.

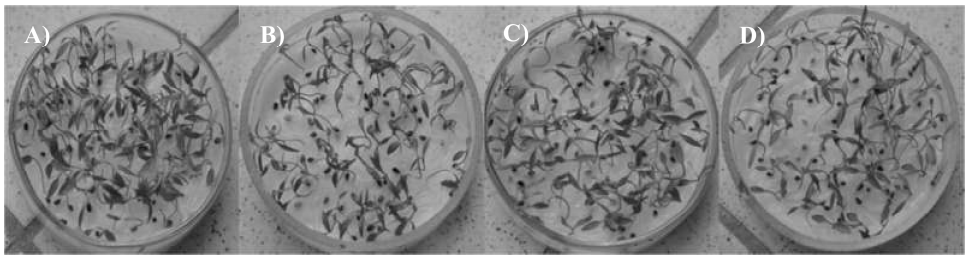

Fig. 10. Sample dishes with Osborne seeds 21 days after sowing
A) Control sample, B) 15 min stimulation, C) 30 min stimulation, D) 60 min stimulation

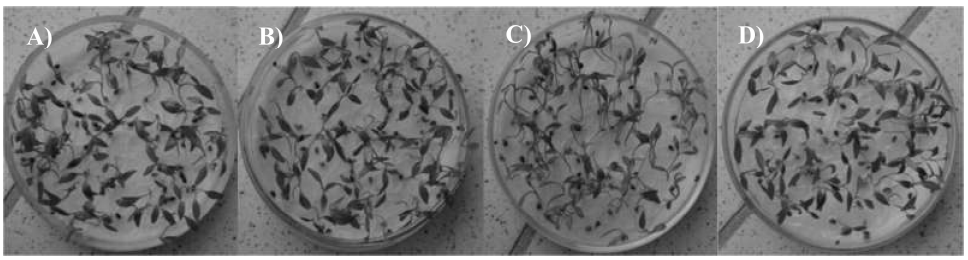

Fig. 11. Sample dishes with Alba seeds 21 days after sowing
A) Control sample, B) 15 min stimulation, C) 30 min stimulation, D) 60 min stimulation

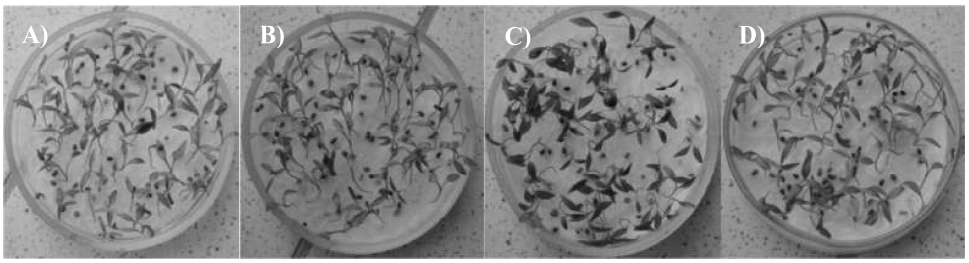

Fig. 12. Sample dishes with Hanácká seeds 21 days after sowing
A) Control sample, B) 15 min stimulation, C) 30 min stimulation, D) 60 min stimulation

CONCLUSIONS

Near-infrared stimulation did not affect the germination energy of all tested varieties of parsley seeds. On the box plot chart (below) one can observe that germination capacity clearly increased for Konika and Alba varieties. This is much less visible for the Hanácká variety (large dispersion value). However, for a the Osborne variety, germination is completely random with respect to exposure time. We believe that we should repeat this experiment with a larger group of studied plants and also include other stimulants (e.g. stimulation with variable and constant magnetic field).

Our results were analysed by the Kruskal-Wallis (K-W) test for many independent groups. This is a nonparametric test which compares more than two groups with each one another. It is analogous to the variance analysis. This test does not require the fulfilment of the assumption of the normality of the obtained results. The idea of this test is the measurement of the location summary one-point statistics in all analysed groups. The whole sample is ranked (all groups are combined) and then the Kruskal-Wallis test statistic is constructed. This statistic is a measure of the deviation of sample ranks from the average value of all ranks. The distribution of statistics is derived with the less restrictive assumption that we compare at least three groups, all of which have a population of at least 3.

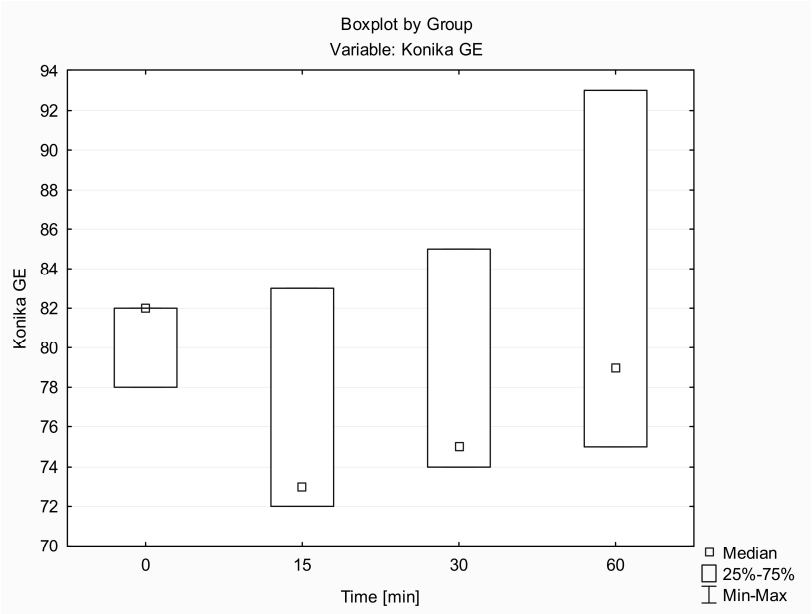

Fig. 13 Boxplot for Konika, Germination energy

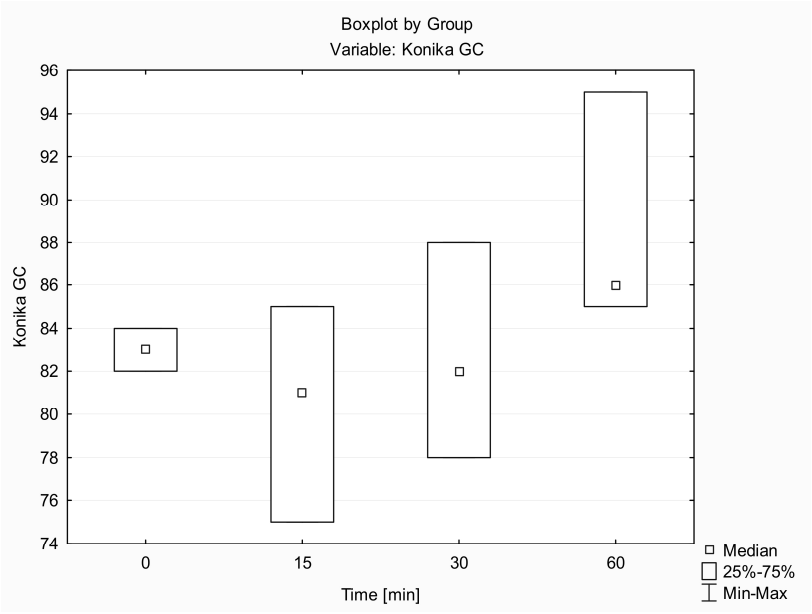

Fig. 14 Boxplot for Konika, Germination capacity

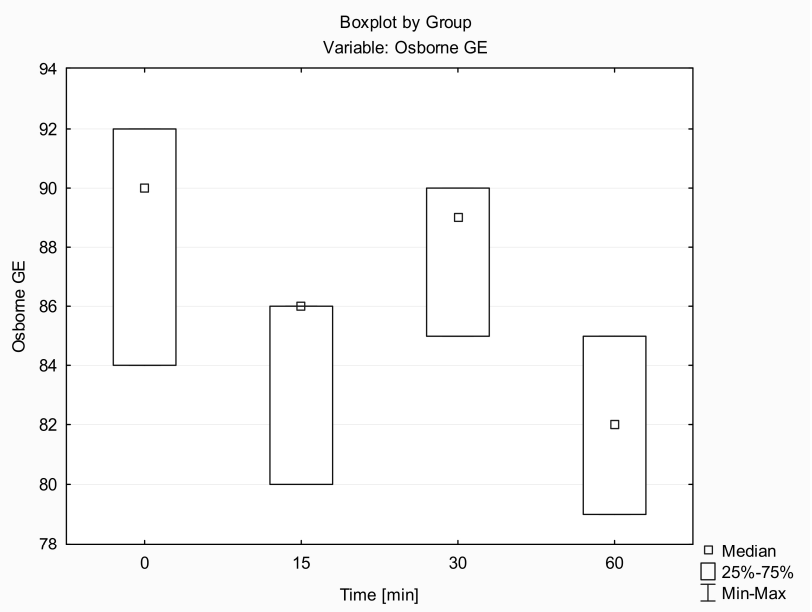

Fig. 15 Boxplot for Osborne, Germination energy

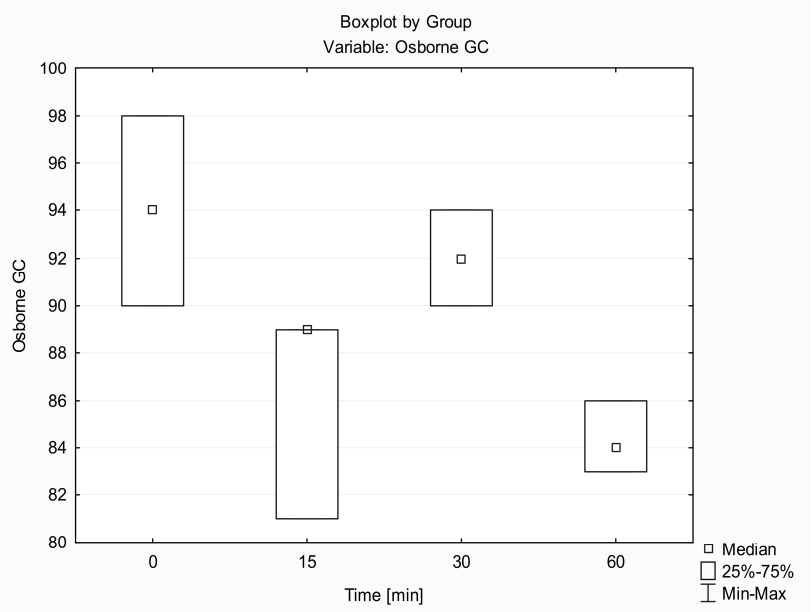

Fig. 16 Boxplot for Osborne, Germination capacity

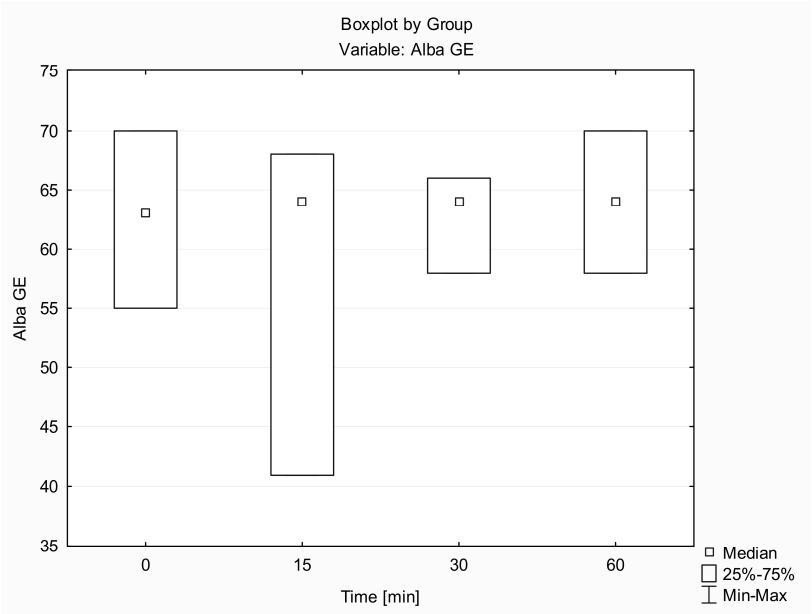

Fig. 17 Boxplot for Alba, Germination energy

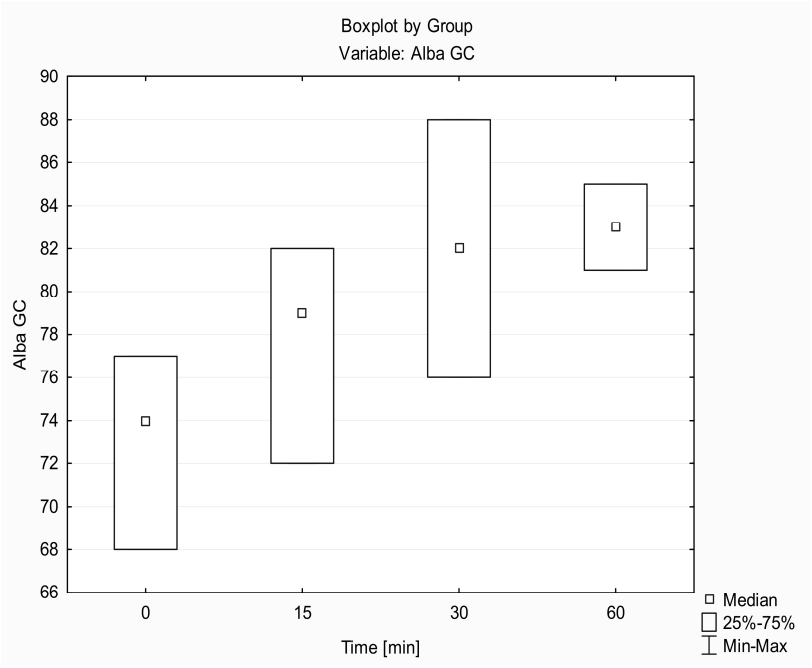

Fig. 18 Boxplot for Alba, Germination capacity

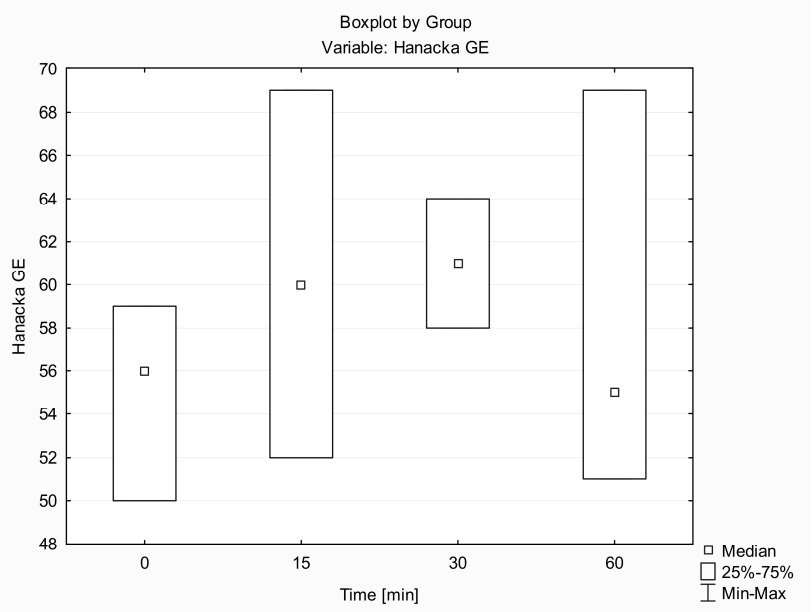

Fig. 19 Boxplot for Hanácká, Germination energy

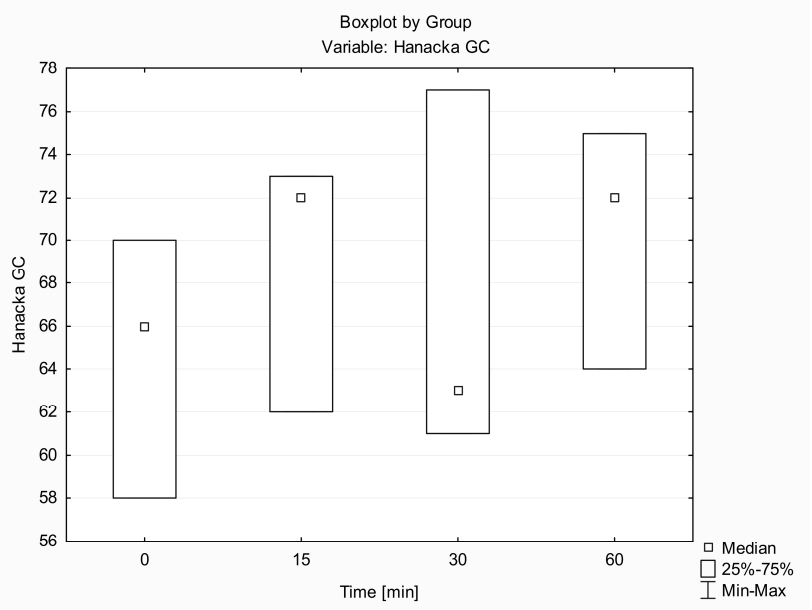

Fig. 20 Boxplot for Hanácká, Germination capacity

In the K-W test, the significance of the effect of NIR exposure time on germination energy was analysed (Figs. 14, 16, 18 and 20), as well as the capacity

(Figures 15, 17, 19, 21), the significance level was 0.05. The results of the test are presented in the following charts (median - first and third quartile - minimum and maximum). Explanation of the abbreviations used: GE - germination energy; GC - germination capacity.

The results of statistical tests confirm our earlier observations. We can see that NIR stimulation gives different results depending on the parsley varieties and the exposure amount. We cannot generally say that this stimulation affects the parsley seeds in one way. However, in most cases, the beneficial effect of NIR on the seeds of selected parsley varieties can be noticed. The germination capacity of Konika and Alba varieties after biostimulation increases (statistically significant). This is also evident for the Hanácká variety, but definitely less clearly (there is a large variation of this value). However, for the Osborne variety, GC is completely random compared to the time of exposure. In the case of germination energy, such effective results cannot be observed (no statistically significant differences between the examined groups). On basis of the results we can find some effect of near-infrared stimulation for GE, however, it is not possible to form unambiguous cognitive conclusions. In some cases, you can even observe the progress of changing this parameter as the exposure time increases (e.g. the Konika variety).

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STUDY OF THE EFFECT OF LOW TEMPERATURES AND CALCIUM CHLORIDE TREATMENT ON THE GERMINATION OF IRANIAN AND EUROPEAN BARLEY CULTIVARS

ABSTRACT

Low temperature stress is one of the limiting factors of seed germination. In order to investigate the effect of low temperatures on germination of barley cultivars, identification of traits related to low temperature stress at germination stage and the effect of calcium chloride on these traits, 44 Iranian and European barley cultivars were evaluated in a factorial experiment within completely randomized design with 3 replications in the Laboratory of Plant Physiology, Agronomy and Plant Breeding department, Razi University. The first factor was 44 Iranian and European barley cultivars, the second factor included four temperature (0, 5, 10 and 20°C), and the third factor was the use of calcium chloride (10 mM) and its non-use (distilled water). Analysis of variance showed that there was a significant difference between cultivars for all traits except root length and seed vigor. Applying calcium chloride treatment at a concentration of 10 mM did not significantly affect the traits under the studied temperatures. Reducing temperature from 20°C to 10°C and 5°C reduced root length, shoot length, coleoptile length, root number, coefficient of velocity of germination, seed vigor and promptness index. The results of correlation analysis showed that there was a significant positive correlation between promptness index with average velocity of germination, coefficient of velocity of germination and seed vigor, germination percentage and root number in all studied temperatures. There was little differentiation between Iranian and European cultivars by both cluster and discriminant analysis.

Key words: barley, correlation, germination, low temperature stress

INTRODUCTION

Barley as an agronomic plant compatible with drought stress and tolerant to adverse environmental conditions and possessing characteristics such as green grazing in the tillering, grain extraction and its use in food industry, has a special place in the agricultural systems of the arid regions of the world, including Iran (Rezaikalu *et al.*, 2012). Barley is cultivated in many parts of the world due to its high resistance to environ-

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mental stresses and less need for moisture and adaptation to the environment (Behnia, 1996). Barley is planted in an area of 1.8 million hectares in Iran, of which 60% is devoted to rainfed areas. Most of the rainfed lands are located in cold and humid areas. In cold regions, in addition to cold and drought stresses, most of the years, due to delays in precipitation in early autumn, seedling emergence due to cold occurrence is difficult causes decreasing the growth period and ultimately decreasing yield (Abdolrahmani *et al.*, 2011). Low temperature stress is one of the limiting factors for plants germination. Germination plays an important role in grain quality and malt quality (Chloupek *et al.*, 2003). Germination is a trait that varies greatly among populations (Baskin and Baskin, 1998).Cultivars with fast germination properties are more likely able to absorb more water and are more suitable for rainfed conditions due to resistance to winter cold (Rastegar, 1992). Calcium is an essential nutrient, and it plays an important role in the activation of metabolic activities, such as membrane stabilization, signal transduction through the second transducer, membrane preservation, and control of the transfer of ion particles and of the activity of enzymes in counteracting the conditions of environmental stresses (Arshi *et al.*, 2006). When the plant is exposed to environmental stresses, including low temperature stress, calcium can withstand stress by regulate many physiological and cellular reactions (Hirschi, 2004). In a study by Perine *et al.* (2008), in order to increase the rate of germination and increase hormonal activity, instead of using hormones, NaOH, $Mg(OH)_2$, $Ca(OH)_2$ and $NaHCO_3$ was used. The results showed that calcium hydroxide was effective in increasing germination by 60-66%. Sedaghathoor *et al.*, 2015, to study the germination rate of seeds of three species of grass (*Lolium perenne*, *Poa pratensis*, *Cynodon dactylon*) used calcium chloride (2%). Calcium chloride treatment alone has had covert not a significant effect on the traits, but the effects of the type of grass and calcium chloride on the average daily germination had the most effect.

The aims of this study were to investigate the effect of low temperatures stress on germination of Iranian and European barley cultivars, to identify the traits related to tolerance to low temperatures in germination stage and to investigate the possible effect of calcium chloride on germination acceleration and other growth parameters of seedlings under low temperatures stress.

MATERIALS AND METHODS

In order to investigate the effect of low temperatures on germination of barley cultivars, identification of traits related to low temperature stress at germination stage and the effect of calcium chloride on these traits, 44 Iranian and European barley cultivars were evaluated in a factorial experiment within completely randomized design with 3 replications in the Laboratory of Plant Physiology, Agronomy and Plant Breeding department, Razi University. The first factor was 44 Iranian and European barley cultivars, the second factor included four temperature (0, 5, 10 and 20°C), and the third factor was the use of calcium chloride (10 mM) and its non-use (distilled water). Iranian cultivars recieved from Kermanshah Agricultural and Natural Resources Research Center and European cultivars seeds recieved from the Genomics and Post Genomics Institute (CRA-GPG) in Fiorenzola, Italy. Table 1 shows the name, source and some of the characteristics of the studied cultivars. Cultivars are named from 1 to 44.

Table 1

Proprties of studied barley cultivars

Code	Cultivar name	Pedigree	Origin
1	ALIMINI	FIOR 2551 x Federal	European
2	RODORZ	Baraka x Gotic	European
3	SFERA	((Katy x HJ54/30) x Igri x Arda) x (Tipper x Sonja)) x Amillis	European
4	ALFEO	Tipper x Igri	European
5	SIRIO	FIOR 2136 x Arco	European
6	ARDA	Igri x HJ 51-15-3	European
7	PONENTE	(Vetulio x Arma) x Express	European
8	ALDEBARAN	Rebelle x Jaidor	European
9	TREBBIA	selection from Fior Synt 3	European
10	ZACINTO	IABO 329 x Arda	European
11	ALISEO	(Plaisant x Gerbel) x Express	European
12	ALCE	(Tipper x Igri3) x [(Tipper x Alpha)x(Sonja x Wb117/18)]	European
13	PARIGLIA	Airone x Arco	European
14	AQVIRONE	FIOR 5186 x Naturel	European
15	ASTARTIS	(IABO x Arda3) x Amillis	European
16	AIACE	FO 1078 x FO 1638	European
17	COMETA	PO202.169 x FO 3358	European
18	NURE	(FIOR 40 x Alpha2) x Baraka	European
19	AIRONE	Gitane x FIOR 763	European
20	SCIROCCO	FIOR 1000 x Express	European
21	MARTINO	FIOR 3007 x Federal	European
22	EXPLORA	[(Onice\Arma\\Onice\Mirco\\\Jaidor) x (Plaisant\Jaidor\Express)] x Gotic	European
23	VEGA	Rebelle x FIOR 1341	European
24	PANAKA	Amillis x Diadem	European
25	Sahra	L. B. LRAN/ Una8271// Giorias"s" Com	Iranian
26	Yusef	Lignee527/chn-01//Gustoe/4/Rhn-08/3/Deir Alla 106//DI71/strain 205	Iranian
27	Denmarck	Denmark55	Iranian
28	Zarjoo	1-28-9963	Iranian
29	Makoie	Star	Iranian
30	Karoon	Strain- 205	Iranian
31	Mahoor	Wi2291/Wi2269//Er/Amp	Iranian
32	Fajr30	Lignee131/ Gerbet//Alger- Ceres/ jonoob	Iranian
33	Sararood	Chicm/An57//Albert	Iranian
34	Gorgan4	Herta	Iranian
35	Jonoob	Gloria" s"/ Copal"s"	Iranian
36	Reihani	Rihane-03/4Alanda/ILignee527/Arar/3/Centinela/2*	Iranian
37	Nimrooz	Trompillo, CMB74A-432-25B-1Y-IB-IY-OB	Iranian
38	Nosrat	Karoon/Kavir	Iranian
39	Afzal	Chahafzal	Iranian
40	Aras	Arumir	Iranian
41	Ansar	Not Clear	Iranian
42	Nader	Not Clear	Iranian
43	Local	Not Clear	Iranian
44	Sararood1	Not Clear	Iranian

From each barley, for each experimental unit, 20 healthy seeds were selected and disinfected according to the following steps: First, the seeds were washed with distilled water and then disinfected with 70% alcohol for 1 minute and 3% hypochlorite for 3 minutes. Then, three times washed with distilled water for 1 minute, 3 minutes and 5 minutes. Seeds were then dressed with mancozeb fungicide (at a rate of 2 g a.i/kg) and cultured in Petri dishes under sterile conditions. The germinated seeds were counted daily for 10 days. The traits were measured based on the average of 10 seedlings including root length (cm), number of root, shoot length (cm), coleoptile length (cm) and the following traits:

$$GP = \frac{Ni}{S} \times 100$$

where
GP – Germination percentage
Ni – Number of germinated seeds
S – Total number of seeds

$$AVG = \frac{\sum Nt}{\sum t}$$

where
AVG – Average Velocity of Germination in day / number:
ΣNt – Total number of germinated seeds at time
Σt – Total time (day), (Salehzade *et al.*, 2009)

$$CVG = \frac{N_1 + N_2 + \cdots + N_x}{N_1 \times T_1 + \cdots + N_x \times T_x} \times 100$$

where
CVG – Coefficient of Velocity of Germination:
N_1 to N_X – the number of seeds germinated from the first day to the end of the test.
T_1 to T_x are the time of counting
This index is a characteristic of the seed germination rate (in day)., calculated from the following equation; (Scotte *et al.*, 1984)

$$PI = nd_2(1.0) + nd_4(0.8) + nd_6(0.6) + nd_8(0.4) + nd_{10}(0.2)$$

where
PI – Promptness Index:
nd2, nd4, nd6, nd8 and *nd10* – the number of germinated seeds on the second, fourth, sixth, eighth and tenth day (Bouslama and Schapaugh, 1984).

$$SV = (SL + RL) \times GP$$

where
SV – Seed Vigor:
RL: Root length,
SL: Shoot length,
GP – Germination percentage; (Hamidi *et al.*, 2009)

$$PCT = \frac{X_n - X_s}{X_n}$$

where
PCT– percentage change of traits
Xn – the mean of trait in control conditions
Xs – the mean of traits in the stress conditions

Statistical analysis

Data were analyzed based on based on a 44 × 2 × 4 factorial experiment within completely randomized design. Mean comparisons were determined with Least Significant Difference (LSD) test by the SAS software ver.9.2. Pearson's correlation coefficients between measured traits evaluated in all tempratures level and cluster analysis based on the Euclidean distance square using Ward's method were done by SPSS software (Ver. 16.0.1, SPSS Inc).

RESULTS

Analysis of variance

None of the studied cultivars germinated at 0°C temperature in all three replications, so the temperature level of 0°C was eliminated from the statistical analysis. Analysis of variance of germination traits in 44 barley cultivars showed that there was a significant difference between cultivars for all traits except root length and seed vigor index (Table 2). The mean comparisons of 44 barley cultivars for the studied traits were done by using the least significant difference test (LSD). Considering the significance of the two and three way interactions for the studied traits, except for root length and seed vigor index, LSD test was performed only on these interactions, some of which are mentioned. Comparison of the significant interaction effect of calcium chloride and temperature for measured traits (Table 3) showed that at 5° C, except for the coleoptile length, other traits in the calcium chloride treatment decreased compared to distilled water. At 10°C, no significant difference was observed in the measured traits between calcium chloride and distilled water treatments. Only a significant decrease for coefficient of velocity of germination in distilled water treatment compared to calcium chloride was observed at 20°C (Table 3). The comparison

of the mean of temperature effect (Table 4) for root length and seed vigor index indicated significant differences in these traits at 20°C compared to 10°C and 5°C.

Table 2

Analysis of variance for germination related traits in 44 barley cultivars

		Mean squares				
Source of variations	DF	CL [cm]	SHL[cm]	RL [cm]	GP	RN
Cultivar	43	2.763**	15.129**	1541.6965^{ns}	4461.55**	4.771**
$CaCl_2$	1	2.371**	7.99216^{ns}	2481.009^{ns}	575.28**	0.0960^{ns}
Temperature	2	1197.3**	5918.56**	9622.65**	50475.66**	187.40**
$CaCl_2$ × Cultivar	43	0.8073^{ns}	5.428^{ns}	1490.4792^{ns}	212.751**	0.260^{ns}
Cultivar × Temperature	86	2.76**	16.61**	1565.1858^{ns}	2509.57**	3.11**
$CaCl_2$ × Temperature	2	2.371**	2.266^{ns}	2095.3263^{ns}	654.64**	0.004^{ns}
Cultivar ×Temperature × $CaCl_2$	86	0.807**	4.44^{ns}	1492.3237^{ns}	180.47^{ns}	0.337^{ns}
Error	528	0.57	4.23	1439.31	122.28	0.27
Source of variations	DF	PI	SV	CVG	VG	
Cultivar	43	133.543**	14207020^{ns}	0.0198**	2.753**	
$CaCl_2$	1	15.4448*	24573048^{ns}	0.001^{ns}	0.3140*	
Temperature	2	1457.13**	141361556**	2.184**	31.400**	
$CaCl_2$ × Cultivar	43	4.913**	12353878^{ns}	0.0020^{ns}	0.1429**	
Cultivar × Temperature	86	77.649**	14355716^{ns}	0.0205**	1.552**	
$CaCl_2$ × Temperature	2	17.904**	15985379^{ns}	0.0127**	0.420**	
Cultivar ×Temperature × $CaCl_2$	86	3.852*	12366553^{ns}	0.0025^{ns}	0.109*	
Error	528	3.02	11657824	0.0024	0.077	

Table 3

Results of mean comparison of interaction effect of temperature and $CaCl_2$ or significant traits in barley cultivars

Variant	Temperature [°C]	Coleoptile length [cm]	Average germination velocity	Germination velocity coefficient	Promptness Index	Germination percentage [%]
Distilled water	5	0.109 d	1.451 c	0.18 cd	7.365 c	57.917 c
$CaCl_2$	5	0.047 d	1.322 d	0.17 d	6.486 d	52.765 d
Distilled water	10	1.616 c	2.029 a	0.188 c	10.759 b	81.061 a
$CaCl_2$	10	1.580 c	2.055 a	0.188 c	10.821 ab	82.083 a
Distilled water	20	3.853 a	1.536 b	0.33 b	11.183 a	61.364 b
$CaCl_2$	20	3.524 b	1.519 b	0.347 a	11.162 ab	60.379 bc

Values followed by the same letter in the same column are not significantly different

Table 4

Results of mean comparison for main effect of temperature on root length and seed vigor

Temperature [°C]	Root length [cm]	Seed Vigor
20	12.290 a	1471.5 a
10	3.44 b	461.9 b
5	0.748 c	41.2c

Values followed by the same letter in the same column are not significantly different

Percentage changes in traits at different temperatures compared to 20°C.

Reducing the temperature from 20°C to 10°C and 5°C resulted in a significant decrease in coleoptile length (Table 5). The roots number decreased by about 36.71 % compared to 20°C by reducing the temperature to 5°C. At the temperature of 10°C, the germination percentage and the average velocity of germination increased compared to the temperature of 20°C, but the reduction of temperature to 5°C reduced these traits. Coefficient of velocity of germination and promptness index decreased at 10°C and 5°C than 20°C.

Table 5

Variation percentage of traits related to germination in 44 barley cultivars in different temperatures compare to 20°C

RL: root length ،SHL: shoot length ،CL: coleoptile length ،RN: root number ،GP: germination percentage,

PI [%]	SV	CVG [%]	AVG	GP [%]	RN	CL [cm]	SHL [cm]	RL [cm]	Temperatures [°C]
3.424	68.610	44.490	-33.649	-34.008	4.496	81.618	81.618	58.070	10
38.011	96.656	48.339	9.252	9.085	36.717	99.129	99.129	90.901	5

AVG : average velocity of germination ،CVG: coefficient of velocity of germination ،SV: seed vigor ، PI: promptness index

Correlation analysis

Pearson correlation analysis for all three temperatures are presented in Table 6. Correlation analysis of traits showed that there is a positive and significant correlation between root length and all traits measured at 5°C. Root number at 5°C had a positive and significant correlation with all traits except shoot and coleoptile length. There was a positive and significant correlation between shoot length and coefficient of velocity of germination at 5 and 10°C. Celoplite length showed a positive and significant correlation with root length, shoot length and seed vigor at 5°C. At 10°C, coleoptile length had significant positive correlation with coefficient of velocity of germination, shoot length and seed vigor and at 20°C with all traits except root length and seed vigor. Germination percentage showed positive and high correlation with seed vigor at all three temperatures. There was a positive and significant correlation between promptness index with coefficient of velocity of germination, average velocity of germination, seed vigor index, germination percentage and root number in all three temperatures.

Table 6

Correlation matrix between measured traits related to germination in 44 barley cultivars under different temperatures

Traits	Temp .	RL	SHL	CL	RN	GP	AVG	CVG	SV	PI
	5°C									
RL	10°C									
	20°C									
	5 °C	.431**								
SHL	10°C	.074								
	20°C	.101								
	5°C	.431**	1.000**							
CL	10°C	.074	1.000**							
	20°C	.164	.684**							
	5°C	.552**	.231	.231						
RN	10°C	.509**	.277	.277						
	20°C	.110	.776**	.799**						
	5 °C	.428**	.046	.046	.662**					
GP	10°C	.120.	.117	.117	.267					
	20°C	.205	.325*	.506**	.607**					
	5 °C	.428**	.046	.046	.662**	1.000**				
AVG	10°C	.120	.116	.116	.267	1.000**				
	20°C	.204	.313*	.496*	.596**	1.000**				
	5 °C	.626**	.230	.230	.430**	.461**	.460**			
CVG	10°C	.267	.326*	.326*	.546**	.325*	.325*			
	20°C	.065	.776**	.754**	.905**	.617**	.605**			
	5°C	.867**	.569**	.703**	.665**	.710**	.709**	.617**		
SV	10°C	.105	.703**	.703**	.244	.301*	.302*	.292		
	20°C	.982**	.119	.195	.166	.369*	.369*	.129		
	5°C	.509**	.091	.230	.670**	.983**	.983**	.587**	.770**	
PI	10°C	.203	.230	.230	.436**	.918**	.917**	.668**	.306*	
	20°C	.191	.324*	.493**	.603**	.997**	.997**	.632**	.356*	

Cluster analysis

The cluster analysis for the data obtained from the germination test was performed using the Ward method based on the Euclidean distance square (Fig. 1). The results of the discriminant analysis divided the dendrogram into two groups and did not differentiate between Iranian and European cultivars (Table 7). The mean of measured traits in each cluster is shown in Table 8. The first cluster consists of 8 Iranian cultivars and 15 European cultivars and the second cluster consists of 12 Iranian varieties and 9 European cultivars. The first cluster had the highest mean for all studied traits (Table 8).

Fig 1. Cluster analysis of 44 barley cultivars based on traits related to germination using Ward method and square Euclidean distance

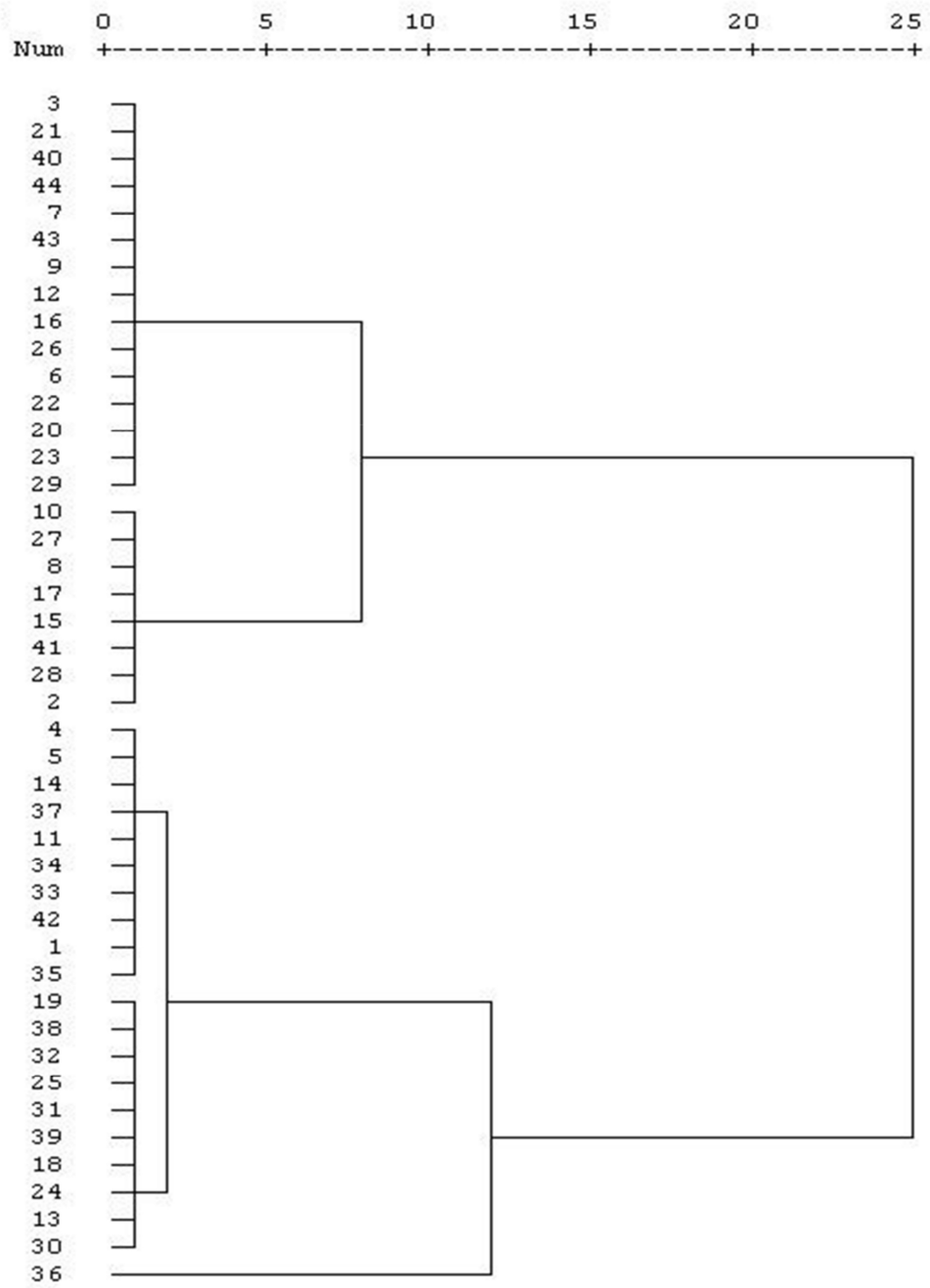

Table 7

Discriminant analysis for grouping 44 barley cultivars based on traits related to germination

Predicted groups			Groups result from cluster analysis	Percentage
Total	2	1		
21	0	21	1	100
23	23	0	2	
100	0	100	1	100
100	100	0	2	

Table 8

Mean of measured traits of 44 barley cultivars in two clusters

Standard deviation ± Mean					Number of cultivars	Cluster
PI	SV	CVG [%]	AVG	GP [%]		
1.27 ± 11.92	12.26 ± 623.30	0.08 ± 0.24	0.15 ± 1.98	6.23 ± 79.36	23	1
1.84± 7.53	179.52 ± 405.56	0.03 ± 0.22	0.26 ±1.34	10.93± 53.65	21	2
Standard deviation ± Mean					Number of cultivars	Cluster
RN	CL [cm]	SHL [cm]	RL [cm]			
0.29 ± 3.88	0.24 ± 1.39	1.87 ± 3.97	1.33 ± 7.12		23	1
0.56 ± 3.39	0.44 ± 1.08	1.14± 3.47	1.21 ± 4.01		21	2

DISCUSSION

The effect of low temperature stress on reducing plant growth is one of the clearest response of plants. Analysis of variance showed that there was a significant difference between cultivars for all traits except root length and seed vigor. Applying calcium chloride treatment at a concentration of 10 mM did not significantly affect the traits under the studied temperatures. Askarian (2004) investigated the effect of CaCl2 on germination of two rangelands species namely Kochia prostrate and Elymus junceus, reported that with increasing CaCl2, germination decreases and even reaches zero. The results showed that the root and shoot length under low temperature stress are accompanied by a decrease, which is consistent with the results of Ghorbani *et al.*, 2009. Abbasal-Ani and Hay, 1983 reported that the growth rate of root and shoot in barley, oat, rye and wheat at low temperature (5 ° C) was low and at high temperature (15 and 25 ° C) is fast. It has been reported that at lower temperatures, the rooting of plants and roots grow decreases (Akbaraghdami *et al.*, 2013). Macduff and Wild (1986) reported that the length and number of roots in germinated barley increased by 27 times, with increasing temperature from 3 to 25°C, after 20 days. The sensitivity of germination percentage and average velocity of germination were lower than other traits, so that at the temperature of 10°C, even the germination percentage and the average velocity of germination increased

compared to the temperature of 20°C , but the reduction of temperature to 5°C reduced these traits. Mei and Song, 2010 reported the optimum temperature for the germination percentage in barley is 5-20°C. In the study conducted by Dinari and Meighani, 2014, the effect of cold stress on seed germination and growth of *Hordeum spontaneum* L (root and shoot length and weight) were studied. Reducing the temperature reduced seedling growth, but seed germination was more tolerant to cold stress than seedling growth. Klos and Brummer (2000) stated that the temperature of the environment determines the success of germination and seedling growth, and affects the capacity and velocity of germination. Particularly temperatures below the optimum can cause poor seed germination. Cultivars with fast velocity of germination are more likely to able to absorb water and adapt to snvironment and, due to their winter resistance, are more suitable for rainfed conditions (Rastegar, 1992).

One of the most sensitive traits to low temperatures was seed vigor, which decreased by about 96.65% at 5°C. Due to changes induced by low temperatures, root capacity decreases for water absorption and ultimately plant growth reduces (Akbaraghdami *et al.*, 2013). Root length showed significant positive correlation with all the traits measured at 5° C. Root length can be an important indicator for predicting the emergence of seedling in the field and it is also considered as the primary index of growth and development of seedlings and its changes as an indicator of seedling vigor are analyzed (Bagheri *et al.*, 2012).

Root number at 5°C showed positive correlation with most of traits. Most cold-resistant plants, including barley, when exposed to low temperatures, show signs of water stress (low water potential and leaf inflammation), which is known as drought stress due to frostbite (Ghorbani *et al.*, 2009. Creating a deep and widespread root system as a result of an increase in root number and length with fast growth rate resulted resistance to stress (Kafi, 1997). Tikonov (1973) studied the roots number in the germination stage in 40 wheat cultivars, and observed that the varieties with the highest roots number at germination time had the highest yield under rainfed conditions. Positive and significant correlation between shoot length and coefficient of velocity of germination at 5 and 10°C observed. Cultivars with high coefficient of velocity of germination and shoot length when exposed to cold stress, have better tolerance and better growth (Akbaraghdami *et al.*, 2013).

Celoplite length showed a positive and significant correlation with most of traits at all tempratures. The importance of the coleoptile length in rapid emergence, early deployment and plant diameter, which protects the plant from environmental damage, such as cold and drought, has been reported (Shakeri *et al.*, 2013). Positive and high correlation of germination percentage with seed vigor at all three temperatures indicated seeds that have better seed germination under stress conditions have stronger seedlings (Jajarmi, 2012). Promptness index showed correlation with coefficient of velocity of germination, average velocity of germination, seed vigor index, germination percentage and root number in all three temperatures. In plants such as barley, which are planted early in autumn, less germination time can result in faster seedling growth, and consequently rosseting and resistance to cold weather (Jajarmi, 2012).

Cluster analysis classified cultivars with more desirable germination characteristics under all studied temperatures into separate group. The cultivars in this cluster can be considered as resistant to low tempreratures during germination and seedling growth stages. The varieties grouped in the first cluster can be used in breeding programs for improvement of parameters related to germination in low temperatures. However, in order to be more reliable, the test should be repeated in a range of low temperatures and in field conditions.

CONCLUSION

- In general, the temperature decrease reduced the characteristics related to germination in the barley cultivars. The cultivars with desirable root number and length, shoot and coleoptiles,
- Length showed better germination characteristics under low temperature conditions. Application of calcium chloride treatment with a concentration of 10 mM did not significantly influence the traits under the studied temperatures. It would seem that other concentrations should be considered. Cluster analysis classified cultivars with more desirable germination characteristics under all studied temperatures into separate group. Considering the importance of the ability of cultivars to face low temperature stress at the germination stage, it is recommended that the cultivars in this group be used for further studies and test confirmation.

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DETERMINATION OF OPTIMUM CONCENTRATION AND TIME PRIMING OF STEVIA SEED WITH BORIC ACID ($H_3BO_{3)}$ MICRONUTRIENT

ABSTRACTS

In order to determine anoptimal duration and concentration of priming of stevia seed with boric acid for improving germination, an experimental factorial completely randomized design with three replications was conducted in the laboratory of Seed Science and Technology, Shahed University of Tehran in 2015. The first factor was different concentrations of boric acid (zero, 0.5, 1, 1.5 and 2 percent) and the second factor of priming time (0, 8, 16, 24 and 32 hours). Effects of concentration and duration of priming with Boric acid was significant on germination percentage, germination mean time, germination rate, germination energy, germination uniformity, germination mean daily, germination daily rate, germination value, seedling length and seed vigor index. The highest germination percentage, germination rate, germination energy, germination mean daily germination value and seed vigor index was 24 hours priming and the highest germination percentage, germination rate, germination potential, germination value and seed vigor index in priming of 2% Boric acid were obtained. Germination uniformity highest in 24 hours at 1% concentration priming of boric acid (5.51). Seedling length in 24 hours at 1.5% concentration priming with boric acid was highest average 1.02 cm. Generally *Stevia* seed priming with boric acid for 24 hours at concentrations of 1.5 to 2 percent had positive effects on germination indexes and seedling growth.

Key words: boron, germination, micronutrients, nutripriming, seed vigor

Abbreviations

GP — germination percentage
MGT — mean germination time
GR — germination rate
GE — germination energy
GU — germination uniformity
MDG — mean daily germination
DGS — daily germination speed
GV — germination value
SL — seedling length
SVI — seedling vigor index

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INTRODUCTION

There are 17 essential elements for idealistic growth and development of plants that are divided into macro- and microelements. In addition to their roles as a cofactor in enzymes and redox reactions, Micronutrient elements have several important and vital actions in plants (Farooq *et al.,* 2012). More importantly, micronutrients are involved in physiologically key reactions of photosynthesis and respiration pathways (Mengel *et al.,* 2001), and lack of those elements limits these vital processes and subsequently reduce productivity. For example, boron deficiency can decrease wheat (Rerkasem and Jamjod, 2004) Chickpea (Johnson *et al.,* 2005) and lentil (Srivastava *et al.*, 2000) productivity. Boric acid is one of the necessary elements for normal growth and development of several different plants (Abdollahi *et al.,* 2012). Reduced productivity because of boron deficiency could be the result of severe disturbance in metabolic reaction involved with this element, like nucleic acid, carbohydrate, proteins and indole acetic acid metabolism, cell wall biosynthesis, phenol metabolism, and preserving plasma membrane integrity and function (Tanaka and Fujiwar, 2008). Functionally boron is related to several different process such as using calcium, cell division, flowering and fruiting, nitrogen and carbohydrate metabolism, disease tolerance, water relationships and specific catalytic reactions (Farooq *et al.,* 2012).

Concentration of priming solution is one of the important factors in seed priming by boron element. For example, research on seed priming with boric acid solution (concentration of 2-20 mM) has been conducted in Russia, and both negative (reduction) and positive (increasing) effects had been reported on germination parameters of different crops like rapeseed, sunflower, soybean, sugar beet, alfalfa, wheat and barley (Shorrocks, 1997 ; Rehman *et al.,* 2012). Significant improvement of germination and primary growth of papaya seedling observed when the seeds were primed with boron solution (2 mg/L) for 6 hours (Deb *et al.,* 2010).

There are different types of priming which being used in priming experiments and they include hydro-, halo-, osmo-, thermos- and hormone priming, and there are different reports on this matter with different plants and different researchers (McDonald, 2000; Iqbal *et al.,* 2012; Rehman *et al.,* 2012; Mirshekari, 2012). Nutri-priming is a relatively new method in which seeds are primed by using micro and macronutrients, has been recently focused on (Rehman *et al.,* 2012; Mirshekari, 2012). Nutrients as fertilizers were used by different methods such as soil application, with irrigation water or foliar fertilization (Rober, 2008). Using nutrients as a seed treatment by coating and seed priming are other methods that could be advantageous (Farooq *et al.,* 2012). Seed priming by using micronutrients (enrichment) has been reported as one of the best ways to beat micronutrient deficiency (Harris *et al.,* 1999). Seed priming with micronutrient elements results in higher rate of water adsorption and metabolism and germination of seeds which consequences could be seen in higher rate of germination, improvement of seedling establishment, higher resistance against stress and pest, and finally higher productivity (Memon *et al.,* 2013).

Stevia (*Stevia rebaudiana* Bertoni), is a perennial, herbal species of *Acetracea* family (Hossein *et al.*, 2008). Stevia plant has a high sweetening property because of steviol glycosides (Singh and Rao, 2005) which firstly are not absorbed by digestive system and so diabetics can use it freely, and secondly, it is not caloric, so it is adequate for fat people who have to care about their daily calorie. Because of its self-incompatibility, stevia pollinate by wind and insects, so the percentage of fertilized flowers and liable is low in this plant and the seeds have low germination percent (Liopa-Tsakalidi *et al.*, 2012). Although, presented studies shows that there is no agreement for the reason of low germination ability of stevia seeds, some researchers introduce self-incompatibility as a reason of weak stevia germination (Oddone, 1997; Purohit, 2008), while some others report that there are no self-incompatibility in this plant (Goettemoeller and Ching, 1999). By the way, weak germination in this plant is an obstacle against large scale planting and results in scarce a high price of effective metabolite of this plant (Raji *et al.*, 2015). In this research, we aim to study the priming with boric acid effects on germination parameters of stevia seeds, and determining best time and favorable concentration of priming solution of this micronutrient element.

MATERIAL AND METHOD

This experiment were conducted in the Seed Science and Technology Laboratory of Agriculture college of Shahed University, using factorial experiment based on a completely randomized design with three replications, in order to determine the adequate time and concentration for stevia seed priming with boric acid in 2015-2016. Priming time and concentration of boric acid solution were 0, 8, 16, 24 and 32 hours and 0, 0.5, 1, 1.5 and 2 percent respectively. Stevia seeds (Bertoni cultivar) which were produced in the agriculture year of 2013 -2014 were purchased from an Indian company (Global Horticulture Products) and disinfected with sodium hypochlorite 10% for 3 min and the rinsed with distilled water. At the end of priming process, the seeds were washed with distilled water and dried for 24 hours in lab. In every petri dish, 25 seeds were placed on Watman paper, the 3 ml water were added and to prevent water evaporation the cover of the petri dishes were fixed and closed by parafim. Seed germination process in growth chamber was controlled on 23±2°C, photoperiod of 18/6 (day/night) and relative humidity of 70±5% (Raina *et al.*, 2013). Counting of germinated seeds starts from day 2 in a specific hour (Liopa-Tsakalidi *et al.*, 2012) and in the end of the experiment, after 11 days, germination percentage (Liopa-Tsakalidi *et al.*, 2012) germination speed (Pagter *et al.*, 2009), mean germination time (Ellis and Roberts, 1981), average germination per day (Hoogenboom and Peterson, 1987), daily germination rate (Steohanie *et al.,* 2005), germination value index (Ghasemi-Golozani and Dalil, 2011), germination energy (Panwar and Bhardwaj, 2005) and seed vigor index (Biradar *et al.*, 2007) were calculated according to the equations in Table 1. Data analysis and comparison of evaluated parameters were done by SAS 9.1 and Duncan multiple range test at 5%, respectively.

Table 1

The computing relation of the parameters studied in the experiment

Parameters	Computing relations
Germination Percentage	$GP = (N \times 100)/M$
Germination Rate	$GR = \sum N_i/T_i$
Mean Germination Time	$MGT = \sum(N_i)/\sum N$
Mean of Daily Germination	$MDG = N/T$
Daily Germination Speed	$DGS = 1/MDG$
Germination Value	$GV = GP \times MDG$
Germination Energy	$GE = M_{cgr}/(N_i \times 100)$
Seed Vigor Index	$SVI = GP \times$ Mean (SL)

N = sum of germinated seeds at the end of the experiment, *M* = total planted seeds, *T* = period of germination, *Ti* = number of days after germination, n = number of germinated seeds in *Ti*, *Mcgr*= maximum cumulative germination percentage, *Ni*= Total seeds sown, *SL*= Seedling Length

RESULTS AND DISCUSSION

Germination percentage

The effect of time period and concentration of boric acid on stevia seeds germination were significant in $P \leq 0.01$ (Table 2). The highest rate of germination among the priming times and different concentration of acid boric, was observed for priming time for 24 hours and the concentration of 2% with the average of 42.66 and 44 percent, respectively (Table 3 and 4). Seed priming with boric acid 2% increase germination rate by 35.72 in comparison with control in stevia seeds. There was a significant and positive correlation at $P \leq 0.01$ between germination percentage and germination rate, germination energy, seedling length, germination value index, germination uniformity and germination value (Table 5). Together, these results indicate that increasing in seed germination percent will lead to improvement of seed germination parameters in this plant, and finally will result in seed vigor index and germination energy . In agreement with our data, Bayat *et al* (2014) showed significant and positive correlation between seed germination and seedling length, seed vigor, germination energy and germination rate. Mirshekari (2012) reported that *Anethum graveolens* L. seed germination with acid boric micronutrient have a significant effect on seed germination and boric acid 1.5 % has the highest rate of germination in this plant. It should be noted that much higher concentration and longer times for seed priming with micronutrients have negative effects on seed germination parameters (Mirshekari, 2012).

Table 2

Summary of variance analysis for effect of times (0, 8, 16, 24 and 32 h) and concentration (0, 0.5, 1, 1.5 and 2 %) of priming with boric acid (H_3BO_3) on Stevia (*Stevia rebaudiana* Bertoni) seed germination indices and growth seedling

		Mean square				
Sources of variance	DF	GP	MGT	GR	GE	GU
Priming Time (PT)	4	283.94**	2.83**	2.32**	0.00007**	1.21**
Priming Concentration (PC)	4	579.94**	0.33*	1.97**	0.00018**	1.49**
PT × PC	16	3.81ns	0.12 ns	0.08 ns	0.00001 ns	0.17**
Experimental error	48	7.21	0.09	0.05	0.000009	0.002
Coefficient of variation [%]		7.41	6.16	11.08	22.09	1.11
Sources of variance	DF	MDG	DGS	GV	SL	SVI
Priming Time(PT)	4	2.34**	0.0043**	1.32**	0.092**	5501874.6**
Priming Concentration (PC)	4	4.79**	0.0006*	2.53**	0.226**	11216896**
PT × PC	16	0.03 ns	0.0002 ns	0.02 ns	0.001**	60269.3 ns
Experimental error	48	0.05	0.0001	0.03	0.0006	52007.1
Coefficient of variation [%]		7.41	6.32	14.83	3.43	7.96

(GP — Germination percentage, MGT — Mean Germination of time, GR — Germination rate, GE — Germination energy , GU — Germination uniformity, MDG — Mean daily germination, DGS — Daily germination speed, GV — Germination value, SL — seedling length, SVI — Seedling vigor index)

Mean germination time

Base on analysis of variance, the priming time and priming concentration of seeds with boric acid have a significant effect on average time required for germination (Table 2). The highest and lowest required time for germination observed in 32 and 8 hours of priming, respectively (5.65 and 4.50, respectively, Table 3). Among different concentration of boric acid, the concentration of 1% have the highest required time for germination which belong to the same group as 1.5 and 2 percent, but have a significant level of difference with the others (Table 4). Average required time for germination has negative correlation and significant difference with germination speed and daily germination speed at $P \leq 0.01$ (Table 5). Seed priming with boron (nutri-priming) have advantageous effects on seed germination, mean time of germination, seedling vigor index and seedling length (Memon *et al.,* 2013).

Table 3

Mean comparison of stevia (*Stevia rebaudiana* Bertoni) seed germination indices under effect of different priming time with Boric acid (H_3BO_3)

Priming time [h]	GP	MGT [day]	GR [seed per day]	GE
0	31.46 d	4.89 b	1.84 c	0.012 c
8	33.60 c	4.50 c	2.19 b	0.012 c
16	38.13 b	4.80 b	2.33 b	0.014 b
24	42.66 a	4.73 b	2.70 a	0.017 a
32	35.20 c	5.65 a	1.72 c	0.013 bc
Priming time [h]	MDG	DGS	GV	SVI
0	2.86 d	0.20 b	0.93 d	2135.5 e
8	3.05 c	0.22 a	1.05 cd	2539.20 d
16	3.46 b	0.20 b	1.36 b	3029.87 b
24	3.87 a	0.21 b	1.69 a	3757.87 a
32	3.20 c	0.17 c	1.15 c	2852.53 c

In each column, means having at least one same letter, are not significantly different according to Duncan's multiple range test ($p \leq 0.05$). (GP — germination percentage, MGT — mean germination time, GR — germination rate, GE — germination energy , MDG — mean daily germination, DGS — daily germination speed, GV — germination value, SVI — seedling vigor index)

Table 4

Mean comparison of stevia (*Stevia rebaudiana* Bertoni) seed germination indices under effect of different priming concentration with boric acid (H_3BO_3)

Priming concentration [%]	GP	MGT [day]	GR [seed per day]	GE
0	28.26 e	4.76 b	1.69 d	0.010 d
0.5	32.00 d	4.78 b	1.98 c	0.011 cd
1	37.06 c	5.12 a	2.09 c	0.013 c
1.5	39.73 b	4.94 ab	2.38 b	0.016 b
2	44.00 a	4.97 ab	2.64 a	0.018 a
Priming concentration [%]	MDG	DGS	GV	SVI
0	2.56 e	0.21 a	0.74 e	1680.80 d
0.5	2.90 d	0.21 a	0.95 d	2318.67 c
1	3.36 c	0.19 b	1.26 c	2992.27 b
1.5	3.61 b	0.20 ab	1.45 b	3590.40 a
2	4.00 a	0.20 ab	1.78 a	3729.87 a

In each column, means having at least one same letter, are not significantly different according to Duncan's multiple range test ($p \leq 0.05$). (GP — germination percentage, MGT — mean germination time, GR — germination rate, GE — Germination energy , MDG — mean daily germination, DGS — daily germination speed, GV — germination value, SVI — Seedling vigor index)

Table 5

Correlation assessment among stevia (*Stevia rebaudiana* Bertoni) seed germination indices and seedling growth under different concentration and time of priming with boric acid (H_3BO_3)

Indices	1	2	3	4	5	6	7	8	9	10
1-GP	1									
2-MGT	0.09 ns	1								
3-GR	0.85**	-0.41**	1							
4-GE	0.99**	0.09 ns	0.85**	1						
5-SL	0.83**	0.20 ns	0.65**	0.83**	1					
6-SVI	0.96**	0.14 ns	0.80**	0.96**	0.93**	1				
7-GU	0.72**	-0.17 ns	0.77**	0.72**	0.68**	0.73**	1			
8-MDG	0.99**	0.09 ns	0.85**	0.99**	0.83**	0.96**	0.72**	1		
9-DGS	-0.11 ns	-0.99**	0.39**	-0.11 ns	-0.22 ns	-0.16ns	0.15 ns	-0.11 ns	1	
10-GV	0.99**	0.08 ns	0.85**	0.99**	0.79**	0.95**	0.71**	0.99**	-0.11ns	1

ns, * and ** — non-significant, significant at 5% and 1% respectively
GP — germination percentage, MGT — mean germination time, GR — germination rate, GE — germination energy, GU — germination uniformity, MDG — mean daily germination, DGS — daily germination speed, GV — germination value, SL — seedling length, SVI — seedling vigor index

Germination rate

The effect of time and concentration of seed priming with boric acid on germination rate was significant at P≤0.01 (Table 2). Seed priming with acid boric for 24 hours increase germination rate by approximately 33 percent in comparison with control (without priming, Table 3). Highest germination rate was observed for seed priming with acid boric 2% with average of 2.64 seed per day (Table 4). Except for mean time of germination, germination rate has positive and significant correlation with other treatments at P≤0.01 (Table 5). Seed priming with micronutrients results in higher rate of water adsorption and consequently beginning of metabolism and seed germination which consequently produce higher germination rate (Rowse, 1995). Aghighi Shahverdi and Omidi (2015) reports that stevia seed priming for 24 hours with gibberellin hormone results in significant increase in germination rate and other germination parameters in the seeds of this plant.

Germination energy

According to the analysis of variance (Table 2) the effect of time period and the concentration of seed priming on *Stevia* seeds germination energy were significant at P≤0.01. Highest germination energy was for 24 hours of seed priming (0.017) and the lowest germination energy was for control (without priming = 0 hour) and 8 hours of seed priming (Table 3). Also, highest germination energy for stevia seeds was for boric acid 2% with mean of 0.018, which show 44.44% increasing in germination energy in comparison to the control (Table 4). Germination energy is one of the most important parameters to evaluate quality and power of the seeds, and

higher germination energy shows higher quality and power of the seeds (Bayat *et al.*, 2015).

Germination uniformity

The effect of priming period, concentration and interplay between them on germination uniformity was significant (Table 2). Studies on the mean of interplay clearly indicate that highest germination uniformity was for combination of 24 hours of priming in boric acid 1%, and the lowest rate of germination uniformity observed at priming for 0 and 32 hours in control concentration of boric acid (Fig. 1). Farooq *et al*. (2006) introduce improvement of α-amylase activity ad increasing of soluble sugar as the reason for homogenous greening and germination of pretreated seeds of rice.

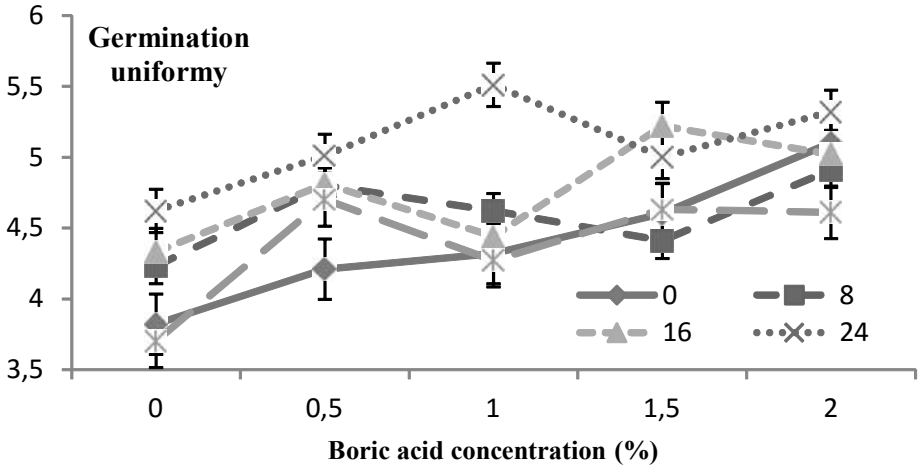

Fig. 1. Mean comparison of stevia seed germination uniformly in concentration×priming time interaction with boric acid (H_3BO_3). Concentration levels=0, 0.5, 1, 1.5 and 2 percentage, Time levels: 0, 8, 16, 24 and 32 h

Mean of daily germination

The effect of time and concentration of priming on mean of daily germination was significant at P≤0.01 (Table 2). Highest and lowest mean of daily germination was for 24 hours period priming and control (zero hour), respectively (Table 3). Among different concentration, the highest rate of it was observed for seed priming with boric acid 2%, which, in comparison with control (concentration zero) show 36.5 increasing in mean of it (Table 4).

Daily germination speed

According to data analysis of variance (Table 2), the effect of priming time period and the concentration of acid boric were significant at P≤0.01and P≤0.05, respectively. Highest speed of daily germination was at 8 hours seed priming with boric acid

(Table 3). Also, except for concentration of 1% which has the lowest mean of daily germination speed, other concentration treatments have insignificant effect compare to each other on this parameter (Table 4). It is reported that low concentrations of boric acid which is involved with activation of enzymes like phosphatase, amylase and so on which are involved in starch metabolism, results in commence of metabolism in seeds and increase daily germination speed by this way (Memon *et al.*, 2013).

Germination value

Data analysis results showed that the effect of time period and concentration of seed priming on germination value parameter for stevia seeds was statistically significant at P≤0.01 (Table 2). Seed priming for 24 hours show highest value of seed germination, and the control time (without priming) had the lowest mean for this parameter (Table 3). Among the experimented concentrations, by increasing of concentration, statistically significant increase was observed for germination value parameter, so that the 0 and 2% concentration have the lowest and highest mean for this parameter (Table 4).

Seedling length

Results analysis of variance (Table 2) showed that the effect of time period, concentration and their interplay (priming time period × priming concentration) on seedling length was statistically significant at P≤0.01. Comparing the mean of the effects of interplay (Fig. 2) showed that the longest (1.02 cm) and shortest (0.44 cm) seedling length observed for 24 hours seed priming with boric acid 1.5% and control (without priming), respectively. Material transport in primed seeds is high because of higher activity of enzymes involved in sucrose metabolism (sucrose synthase, invertase, sucrose phosphate synthase), so these seeds have higher biological power and germinate quicker and produce plumule and radicle, so clearly have higher seedling length (Kaur *et al.,* 2005).

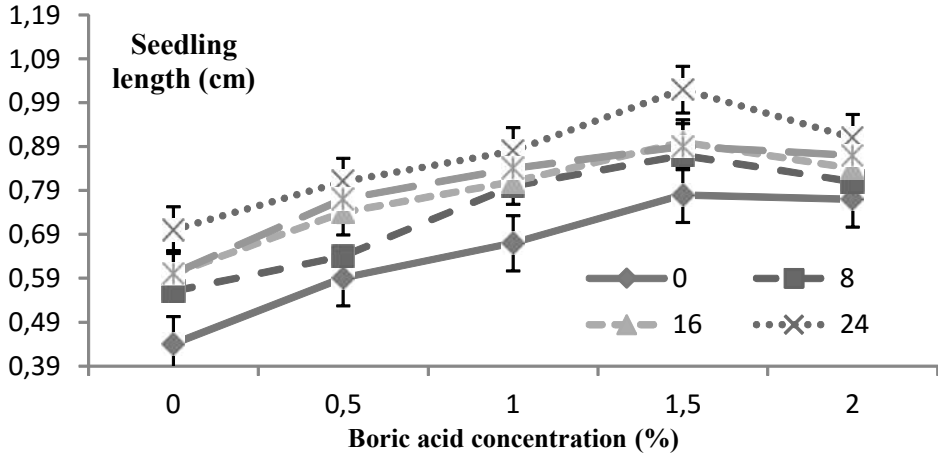

Fig. 2. Mean comparison of stevia seedling length in concentration × priming time interaction with boric acid (H_3BO_3). Concentration levels=0, 0.5, 1, 1.5 and 2 percentage, Time levels: 0, 8, 16, 24 and 32 h

Seed vigor index

According to results, the effect of priming time period and boric acid concentration for priming on seed vigor index, was statistically significant at $P \leq 0.01$ (Table 2). Among the priming periods, 24 hour of seed priming and control have the highest and lowest seed vigor index. Highest seed vigor index gained at boric acid concentration of 1.5 and 2 % which was 53% higher than control (Table 4). Seed vigor index have a positive correlation and statistically significance at $P \leq 0.01$ with germination percentage, germination speed, germination energy , seedling length, germination uniformity, mean daily germination and germination value (Table 5). The importance of seedling vigor for fast establishment and primary growth of medicinal plant in order to competence for receiving water, light and minerals were emphasized in the studies of Tabrizian and Osareh, 2007. Seed priming with boron micronutrients (H_3BO_3) and iron ($FeSO_4$) resulted in increase of seed vigor index in *Anethum graveolens* L. (Mirshekari, 2012).

CONCLUSION

Our results showed that stevia seeds react positively to priming with boric acid, and it improved and increased germination parameters in this plant. In order to enhance seed vigor index and percentage and speed of stevia seed germination, priming with boric acid 1.5 and 2 for 24 hours showed best result in this experiment.

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COLORIMETRIC VS. CHROMATOGRAPHIC ANALYSES OF ALKALOIDS IN LUPIN SEEDS

ABSTRACT

A characteristic trait of lupins is a production of alkaloids, which are a toxic and bitter taste compound of seeds. Due to the lack of fast, sensitive and inexpensive screening techniques to identify and reject high alkaloid plant material, development of suitable tools is important challenges for lupins breeding and seed production. The aim of this study was to compare two alkaloid content estimation methods in *Lupinus angustifolius* L. and *Lupinus albus* L.

During the Wagner's colorimetric test, which is recommended by the UPOV, seed halves were stained on four colors depending on the alkaloid content but only the level of 0.5% – 0.6% showed clear color change. Gas chromatography allowed accurate quantification and qualification of alkaloid content.

Since safe alkaloid content for consumption is 0.02% of seed dry weight, colorimetric method is less useful for dividing lupin cultivars into sweet and bitter, than gas chromatography but can be used as a screening technique.

Key words: gas chromatography; *Lupinus angustifolius* L.; *Lupinus albus* L.; sweet/bitter lupins; Wagner's colorimetric test

INTRODUCTION

The genus *Lupinus* covers four lupin crops: white lupin (*Lupinus albus* L.), narrow-leafed lupin (*Lupinus angustifolius* L.), yellow lupin (*Lupinus luteus* L.) distributed in the Mediterranean basin and Andean lupin (*Lupinus mutabilis* Sweet) originating from the South America. Despite their usage in crop rotation (N-fixation), fertilization, and ornamental, the main usage is as dry seeds for high protein content (up to 45%) and sometimes for oil

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content (up to 14%). Lupin domestication has a short history and the high-yielding cultivars available now are the result of the work of just three generations of breeders (Brummund & Święcicki, 2011) Alkaloids are the antinutritional compounds present in the lupin seeds. Their content can reach 2.88% of the seed dry weight (DW) in narrow-leafed lupin and even 12.73% of the seed DW in white lupin (Kamel *et al.* 2016; Kroc *et al.* 2016) (http://www.igr.poznan.pl/uploads/Lupinus angustifolius-1.pdf; http://www.igr.poznan.pl/uploads/biologiczne%20bazy%20danych%202016/The%20total%20alkaloid%20content%20and%20qualitative%20composition%20of%20%E2%80%A6.pdf)

First, lupins with less alkaloid content were selected and described in twenties/thirties of twenties century (Brummund and Święcicki, 2011; Święcicki *et al.* 2015). Alkaloid content in modern cultivar is e.g. 0.0089% of the seed DW in the narrow-leafed lupin cv. 'Lazur' (Synthesis of results of register trials, 2015). According to Cowling *et al.* (1998) a safe content of alkaloids for consumption is below 0.02% of the seed DW. Guidelines of the International Union for the Protection of New Varieties of Plants (UPOV) (UPOV Guidelines, 2004) divide lupin cultivars into bitter (high-alkaloid) and sweet (low-alkaloid) based on cheap Wagner's colorimetric test and suggest controls, bitter and sweet cultivars, for a given lupin crop (UPOV Guidelines 2004). This test is based on the color reaction of seed cotyledons to the presence of alkaloids. Therefore, the aim of study was to reveal the level of alkaloid content which results in a visible color reaction. Results will be useful for breeding selection and distinction, homogeneity and stability (DHS) description of the cultivars.

MATERIAL AND METHODS

Plant material

For two lupin crops, 20 accessions each with differentiated alkaloid content were selected (narrow-leafed lupin: from 0.007% to 1.50% in seed DW; white lupin: from 0.039% to 3.13% in seed DW), based on the Polish Lupin Collection Database (http://www.igr.poznan.pl/uploads/Lupinus%20angustifolius-1.pdf; http://www.igr.poznan.pl/uploads/biologiczne%20bazy%20danych%202016/The%20total%20alkaloid%20content%20and%20qualitative%20composition%20of%20%E2%80%A6.pdf), UPOV controls: narrow-leafed lupin, bitter cv. 'Azuro' (Wt 95941) and sweet cv. 'Bordako' (Wt 96192) and white lupin, bitter cv. 'Feli' (Wt 95531) and sweet cv. 'Nelly' (Wt 95480) were included in the study. Plants were grown for seed multiplication in the Plant Breeding Station at Wiatrowo (Poznan Plant Breeders Ltd.) during the vegetation season April–August 2015 and harvested in full maturity (water content 13%).

Gas chromatography

From each accession 100 g seeds were sampled. From each sample 10 g was milled for estimation of the total and quantitative composition of alkaloids according to the procedure described by Kamel *et al.* 2016.

Wagner's colorimetric test

The Wagner reagent was prepared in following way. First, 14 g of potassium iodide was dissolved in purified water 7 days before analyzes. Then, 10 g of iodine was added along with purified water up to 100 ml in a dark volumetric flask. The solution was maintained in dark till the analyzes. Before using for the tests, it was diluted five times.

For the colorimetric test, 10 seeds were cut into halves and placed on Petri dishes. Each half of the seed was plunged into the diluted Wagner reagent for 10 seconds and then in purified water for 5 seconds. Then a coloration of the lupin seed halves was observed.

Statistical analysis

The basic statistical characteristics describing quantitative composition of alkaloids in two lupin species were calculated. Also, an analysis of variance for complete randomized design with equal replications was conducted to study substantial differences between means for a percentage share of individual alkaloids.

RESULTS AND DISCUSSION

The quantitative composition of the alkaloids showed clear differences between the lupin crops and was similar to the earlier investigations available in the Polish Lupin Collection (Kamel *et al.* 2016; Kroc *et al.* 2016) (Table 1; http://www.igr.poznan.pl/uploads/Lupinus%20angustifolius-1.pdf; http://www.igr.poznan.pl/uploads/biologiczne%20bazy%20danych%202016/The%20total%20alkaloid%20content%20and%20qualitative%20composition%20of%20%E2%80%A6.pdf). Four major alkaloids (abundance >1% of the total alkaloids) were identified in the narrow□leafed lupin: lupanine, 13-hydroxylupanine, angustifoline and isolupanine; but six were identified in the white lupin: lupanine, 13-hydroxylupanine, multiflorine and angustifoline, albine (mean content 11.88% in seed DW) and 11,12-seco-12,13-didehydromultiflorine (mean content 2.64% in seed DW), both absent in the narrow-leafed lupin (isolupanine is a minor alkaloid, abundance <1%).

The results of the statistical analysis performed to study differences between two species for quantitative content of alkaloids were given in Table 1. This analysis showed a substantially higher mean percentage share of 13-hydroxylupanine, angustifoline and isolupanine in the narrow-leafed lupin, but lupanine and multiflorine in the white lupin.

Table 1

Statistical characteristics of the narrow-leafed lupin (Nar) and white lupin (Whi) alkaloids and results of testing differences between means

Alkaloid	Lupin species	[%Values of total content]		Coefficient of variation [%]	Mean	Differences between means (Nar-Whi)
		Minimum	Maximum			
Lupanine	Nar	11.45	83.00	36.72	49.86	-13.63*
	Whi	30.69	84.93	19.97	63.49	
13-hydroxylupanine	Nar	8.87	65.52	46.67	28.41	18.78*
	Whi	3.08	22.77	52.25	9.64	
Angustifoline	Nar	4.80	25.12	31.25	15.01	11.02*
	Whi	1.11	9.28	50.33	3.99	
Isolupanine	Nar	0.73	14.25	83.81	3.98	3.30*
	Whi	0.30	2.86	92.68	0.68	
Multiflorine	Nar	0.01	2.6	107.38	0.64	-5.15*
	Whi	1.08	17.30	78.32	5.79	

* significant at $p<0.01$

Table 2.

A comparison of two methods of alkaloid content estimation in lupin seeds.

Narrow-leafed lupin			**White lupin**		
Accession number	**Total alkaloid content [% seed DW]**	**Color of seed half**	**Accession number**	**Total alkaloid content [% seed DW]**	**Color of seed half**
96126	0.0015	*	95449	0.0104	*
96225	0.0023	*	95472	0.0250	*
96164	0.0035	*	**95480**[a]	**0.0737**	*
96101	0.0044	*	95496	0.0755	*
96193	0.0062	*	95404	0.1203	**
96131	0.0071	*	95494	0.1337	**
95935	0.0098	*	95422	0.1582	**
96191	0.0146	*	95487	0.2867	**
96195	0.0173	*	95433	0.3649	**
96192[a]	**0.0192**	*	95507	0.4284	**
96114	0.0195	*	95174	0.5427	***
96182	0.0198	*	95168	0.6076	***
96212	0.0296	*	95176	0.6432	***
95927	0.0584	*	95476	0.6774	***
95928	0.0685	*	95486	0.7870	*
96199	0.0760	*	95443	0.9304	***
95916	0.1851	**	95242	1.0264	****
96110	0.4083	**	**95531**[b]	**1.0629**	***
95941[b]	**0.5643**	***	95457	1.1470	****
95719	0.7698	***	95232	1.1833	****
95932	0.7721	***	95208	1.3793	****
95714	0.9774	***	95503	1.7711	****

[a] sweet control [b] bitter control; Half seed color (cotyledons) after Wagner reagent treatment: * yellow, ** dark yellow, *** brown, **** dark brown

The most important aim of this study was to compare the results obtained by two methods, colorimetric and chromatographic (Table 2). The total alkaloid content was differentiated, in the narrow-leafed lupin accessions from 0.0015% to 0.9774% in seed DW (sweet control – 0.1920%, bitter control – 0.5643%) and in the white lupin from 0.0104% to 1.7711% (sweet control – 0.0737%, bitter control – 1.0629%). After the treatment with the Wagner reagent, seed halves (cotyledons) showed four colors: yellow, dark yellow, brown and dark brown. In both lupin crops a clear color change due to the presence of alkaloids is brown color with their content level 0.5%–0.6% in seed DW. So, the Wagner's colorimetric method does not reveal desirable low alkaloid content (0.02% in seed DW maximum), safe for feeding and as such is less useful for dividing lupin cultivars into sweet and bitter. But it can be used in breeding and seed production as an introductory screening technique allowing to select or reject bitter plant material. For further estimation and selection, the chromatographic method must be involved.

CONCLUSIONS

A decrease of alkaloid content in lupin plants is an important aim in breeding. Current challenges for lupins breeding and seed production include the development of fast, sensitive and inexpensive screening techniques to identify and reject high alkaloid plant material. The presented study is focused on comparison of two the most popular alkaloid estimation methods, Wagner's colorimetric test and gas chromatography. Our results show, that Wagner's test can be used only as an introductory screening technique, because clear color change can be observed only on the level of 0.5% – 0.6% of the total alkaloid content. Unfortunately, this is too little sensitivity, since the safe content for consumption amounts 0.02% of seed dry weight. It suggests, that colorimetric method is less useful for distinguishing of sweet lupin cultivars from bitter, than gas chromatography, which clearly determines a qualitative and quantitative alkaloid content. This information would be very helpful for lupin breeders worldwide.

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ASSESSMENT OF TOLERANCE AND STABILITY IN BARLEY (*HORDEUM VULGARE* L.) GENOTYPES AT EARLY SEEDLING GROWTH STAGE UNDER SALINE CONDITION

ABSTRACT

This study was performed to assess several indices for identifying genotypes at early growth stage with the best performance in salinity conditions. In order to evaluate the biomass production of barley genotypes in different levels of salt conditions, an experiment was conducted as factorial arrangement with a completely randomized design with 3 replications. The effect of salinity treatments was studied through an analysis of the dry matter production, yielding results that show significant differences among genotypes. The majority of used tolerance indices indicated that ESBYTM8910, 4 Shori and MBS8715 were the best barley genotypes showing the highest stress resistance for the greatest NaCl concentration. Based on used stability parameters the genotypes MBS8712 and Jo torsh were the most phenotypically stable. Result of cluster analysis revealed that tolerant genotypes showed the least stability based on mostly of stability parameters. In general, results showed the WB7910, ESBYTM8910 and MBS8715 genotypes appeared better than others across the salinity levels.

Key words barley, salt tolerance, stability, tolerance indices

List of abbreviations

ANOVA= Analysis of variance;
CRD= Complete randomized design;
GMP= Geometric mean productivity;
GEI= Genotype*environment interaction;
MP= Mean productivity;
SSI= Stress susceptibility index;
STI= Stress tolerance index;
TOL= Stress tolerance;

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INTRODUCTION

It is estimated that about 15% of the total land area of the world has been degraded by salinization and soil erosion, which are among the major causes of desertification (Mariangela Montemurro, 2015). Abiotic stresses such as drought and salinity are responsible for significant yield losses in barley on a worldwide scale, and yet under severe stress conditions, barley remains to be an important crop used as feed for animals, malt and human food (Katerji *et al.*, 2006). The establishment stage of the crop consists of three parts: germination, emergence and early seedling growth; that are particularly sensitive to substrate salinity (Saboora *et al.*, 2006).

Germination and seedling growth under saline environment are the screening criteria which are widely used to select the salt tolerance genotype. As for better cropping highest plant population is required, which is only possible if seed germination is satisfactory under saline conditions (Nasser *et al.*, 2001). However, the effectiveness of such screens varies but the main emphasis seems to be in balance with a controlled environment, so that the screening techniques are reliable, against the uncertainty of variation in natural conditions in the field (Bernardo *et al.*, 2006).

Salinity impairs seed germination, reduces nodule formation, retards plant development and reduces crop yield (Muhammad *et al.*, 2006). Several researchers have reported the selection of barley genotypes under favourable conditions (Betran *et al.*, 2003). Selection by the aim of stress condition has been highly suggested too. A number of researchers have preferred the mid-way and believe in selection under both favorable and stress conditions (Ashraf *et al.*, 2015). Several selection criteria, such as stress tolerance and mean productivity (Rosielle and Hambling, 1981), stress susceptibility index (Fisher and Maurer, 1978), geometric mean productivity and stress tolerance index (Fernandez, 1992) have been proposed as indicators to identify genotypes with better stress tolerance. Giancarla *et al.* (2012) in evaluating the ability of drought tolerance indices to identify tolerant barley genotypes under laboratory conditions stated these indices may be screened for indirect selection of drought tolerance in the initial stage of the crop growth. As a result, three main strategies have been recognized that plants use to cope with stress: (*i*) *specialization*, the genotype is adapted to the specific environment; (*ii*) *generalization*, the genotype has moderate suitability in most environments; and (*iii*) *phenotypic plasticity*, signals from the environment interact with the genotype and stimulate the production of alternative phenotypes (Fritsche and Borém, 2005).

Phenotypic plasticity is high when compared to the yield stability (Bradshow, 2006). Thus, low plasticity (or high stability) is not always a desirable characteristic because tolerant genotypes generally have moderate productivity, even under ideal growing conditions (Fritsche and Borém, 2005). The economic significance of stability for the cultivation of a genotype was first identified by Roemer (1917), who used the variance across environments as a parameter for yield stability. Using the dynamic concept of stability, Wricke's model (1962) is the simplest method to evaluate the stability. Wricke suggested the ecovalence (W^2_i) concept as the ratio of the interaction sum of squares contributed by each

genotype to the G*E interaction sum of squares. Shukla (1972) proposed the variance component of each genotype across environments as another relevant measure of phenotypic stability. It measures stability rather than performance.

The regression coefficient was introduced by Finly and Wilkinson (1963) as the regression of the mean of i^{th} genotype in jth environment on the mean performance of all genotypes in that environment. A criticism of the use of simple linear regression models is based on the potential non linear pattern of genotype responses to environmental variation. The first proposal to solve this deficiency was presented by Verma *et al.* (1978). They separated the environments into two groups (favorable and unfavorable) and fit a simple linear regression model separately to each part. Eberhart and Russell (1966) suggested using the mean of squared deviation from regression as a measure for stability and a stable genotype is the one has a small deviation from regression mean squares.

The objective of this research was to evaluate the tolerance and phenotypic stability barley genotypes at early growth stage, based on dry matter production, which allows a quick and easy-to-measure screening tool for genotypic differences in salinity tolerance.

MATERIALS AND METHOD

The material used for this study comprised 9 barley promising lines and cultivars i.e.:

STW82153	(A),	WB7910	(F),
MBS8712	(B),	Valfajr	(G),
ESBYTM8910	(C),	MBS8715	(H)
4 Shori	(D),	Jo torsh	(I).
5 Shori	(E),		

Germination tests were carried out at 5 levels of electrical conductivities ($ds \times m^{-1}$):

S1 (control)	=	4.5,	S4	=	13.5,
S2	=	7.5,	S5	=	16.5,
S3	=	10.5,			

Treatments were arranged in a factorial design with 3 replications on the base of a Completely Randomized Design (CRD). Salty solutions were prepared by dissolving NaCl in distilled water at the required concentrations, since this is a common salt that adversely affects plant growth under natural conditions (Yildrim *et al.*, 2011).

First, seeds of each genotype were surface sterilized with 5% sodium hypochlorite solution for 10 min and then rinsed with sterile distilled water three times, to finally be placed on filter paper into 9 cm diameter Petri dishes (25 seeds per Petri dish). In each Petri dish, 5 ml of specific solution was added on alternate days. In

order to avoid salt accumulation, filters were replaced in the same interval of time. Seeds were germinated in an incubator at 25 and were considered to have germinated when the emerging radical expanded to 2 mm (Saboora *et al.*, 2006). After 10 days the effects of salinity treatments were studied by sampling on dry weight of shoot and root as biomass production for each treatment.

The dry weights were measured by drying the shoot and root at 75°C for 48 h, to give a constant weight. Tolerance indices and stability parameters were calculated with this difference that biomass production was replaced with yield.

Tolerance indices of SSI (Fischer and Maurer, 1978), STI and GMP (Fernandez, 1992), MP and GMP (Rosielle and Hamblin, 1981) were calculated. Then six stability parameters were performed in accordance with Eberhart and Russell's (1966) the slop value (b_i) and deviation from regression (S^2_{di}), Roemer's (1917) environmental variance (S^2_{xi}), Wricke's (1962) ecovalance (W^2_i), Shukla's (1972) stability variance (σ^2_i) and Verma model(1978) slop values.

The division of favourable and unfavourable environments was made based on the environmental index that represents the deviation of each environmental mean from the overall mean. Unfavourable environments are those with negative or zero indices and favourable environments have positive indices, so third level of salinity treatments was determined as middle point of tow environments.

All statistical procedures were carried out using the R program (Everitt and Hothorn, 2006). Relationships among variables were determined using Spearman's correlation test, and graphs were created using STATISTICA software. In order to determinate different genotypes and their relationships, cluster analysis was applied. The cluster analysis based on Euclidean distance was performed on the basis of tolerance indices and stability parameters by using the Ward method in the most level of salinity.

RESULT

Analysis of data presented in Table 1. showed that salt stress had adverse effect on seedling growth of barley. There were significant differences between performance barley genotypes and salinity levels. Also, there were significant difference amongst the genotype × salt stress interaction for the biomass production trait. Biomass production was decreased with increasing in salt concentration almost in all barley varieties except the genotype E that showed unchanged biomass production in second level of salinity and even more amount of that in third level of salt concentrations although their differences were not significant (Table 2). Due to significant statistical difference of genotype × salt stress interaction, a selection of genotypes with best performance in a level of salinity based on their production in other levels of salinity will not be possible. The biomass production under control condition was highest for A, C and G, whereas with the highest levels of salinity this was the case for C, D and H (Table 2).

Table 1

Analysis of variance of biomass production data

Parameters	S.O.V				
	Treatment	Genotype(G)	Salt(S)	G×S	Error
DF	44	8	4	32	90
MS	68.87**	53.8**	518**	16.5**	2.5

ns, * and **: Not significant, and significant at the 5% and 1% levels of probability, respectively

Table 2

Statistical comparison of means for genotype biomass production (mgr/plant) by Duncan's multiple range test (α = 0.01)

Genotype	Salt					
	S_1	S_2	S_3	S_4	S_5	
A	27^{ab}	17.67^{hijk}	15.33^{jklmno}	11.67^{opqr}	12.67^{mnopq}	16.8_c
B	24.33^{abcde}	19^{ghij}	15.67^{jklmn}	13.33^{lmnopq}	11.67^{opqr}	16.9_c
C	27^{ab}	18.33^{ghijk}	17.33^{hijk}	15.67^{jklmn}	17^{hijkl}	19.1_{ab}
D	22^{cdefg}	21^{defgh}	20.67^{efgh}	14.67^{klmnop}	15.67^{jklmn}	18.9_{ab}
E	18.33^{ghijk}	18.33^{ghijk}	20^{fghi}	12.67^{mnopq}	11.33^{pqr}	16.4_c
F	24.67^{abcd}	23.33^{bcdef}	21^{defgh}	20^{fghi}	11.33^{pqr}	20.1_a
G	28^{a}	20.33^{fghi}	18.33^{ghijk}	13^{mnopq}	12.33^{nopq}	18.4_b
H	25.33^{abc}	18^{ghijk}	18.67^{ghijk}	20^{fghi}	16.33^{ijklm}	19.6_{ab}
I	20.67^{efgh}	17.67^{hijk}	$15^{jklmnop}$	9.67^{qr}	8.33^{r}	14.1_d
	24.2_a	19.3_b	18.1_c	14.5_d	13_e	

Values followed by different letter(s) differ significantly. Genotypes: STW82153(A), MBS8712(B), ES-BYTM8910(C), 4 Shori (D), 5 Shori (E), WB7910(F), Valfajr(G), MBS8715(H) and Jo torsh(I)

Table 3

Stress tolerance indices values for studied genotypes (S1 vs. S5)

Rank	TOL	MP	GMP	SSI	STI	Yp	Ys
1	D 6.6	C 22.1	C 21.5	D 0.6	C 0.8	G 28.1	C 17
2	E 6.8	H 20.7	H 20.2	H 0.8	H 0.7	C 27.2	H 16.3
3	H 8.9	G 20.3	G 18.7	E 0.8	G 0.6	A 27	D 15.6
4	C 10.2	A19.8	D 18.6	C 0.8	D 0.6	H 25.2	A 12.6
5	I 12.4	D 18.9	A 18.5	B 1.1	A 0.6	F 24.7	G 12.5
6	B 12.6	B 18.2	B 17.1	A 1.2	B 0.5	B 24.5	B 11.9
7	F 13.2	F 18.1	F 16.8	F 1.2	F 0.5	D 22.2	E 11.6
8	A 14.4	E 15	E 14.6	G 1.2	E 0.4	I 20.6	F 11.5
9	G 15.6	I 14.4	I 13	I 1.3	I 0.3	E 18.4	I 8.2

S1= 4.5 ds × m^{-1}, S5=16.5 ds ×m^{-1}

In the highest level of salinity, the best results based on stress tolerance indices (STI, TOL, SSI, MP, GMP) belonged to C, H, E and D genotypes (Table 3). Correlation coefficient tests of stress tolerance indices with Yp and Ys showed various results in different levels of salinity (Table 4).

Table 4

Correlation coefficient of stress tolerance indices with Yp and Ys

Stress tolerance indices	S1 (4.5 ds × m^{-1}) vs. S3 (10.5 ds × m^{-1})						
	TOL	MP	GMP	SSI	STI	Yp	YS
TOL	1						
MP	0.31	1					
GMP	0.19	0.99**	1				
SSI	0.98**	0.22	0.1	1			
STI	0.05	0.95**	0.97**	-0.04	1		
Yp	0.85**	0.76*	0.67*	0.79*	0.56	1	
Ys	-0.69*	0.46	0.56	-0.76*	0.67*	-0.22	1
Stress tolerance indices	S1(4.5 ds × m^{-1}) vs. S5(16.5 ds × m^{-1})						
	TOL	MP	GMP	SSI	STI	Yp	Ys
TOL	1						
MP	0.16	1					
GMP	-0.02	0.98**	1				
SSI	0.89**	-0.26	-0.43	1			
STI	-0.07	0.96**	0.99**	-0.46	1		
Yp	0.62	0.87**	0.76*	0.23	0.73*	1	
Ys	-0.43	0.82**	0.91**	-0.76*	0.92**	0.43	1

* ,**: Significant at the 5% and 1% levels of probability respectively.

The results of five parametric stability statistics are given in Table 5. According to the Eberhart and Russell (1966) model, regression coefficient (b_i) approximating 1.0 coupled with deviation from regression (S^2_{di}) of zero indicate average stability. When this is associated with high mean yield, genotypes have general adaptability and when associated with low mean yield, genotypes are poorly adapted to environments. b_i values above 1.0 describe genotypes with higher sensitivity to environmental change (below average stability), and greater specificity of adaptability to high yielding environments. Regression coefficient decreasing below 1.0 provide a measure of greater resistance to environmental changes (above average stability) and therefore increasing specificity of adaptability to low yielding environments. The genotypes A and G had a higher biomass production and a coefficient values greater than one. These genotypes are sensitive to environmental changes and would be recommended for cultivation under favorable conditions. The genotypes with a b_i value lower than one were D and H that had suitable performance under stress conditions.

Table 5

Stability parameters values of barley genotypes

Geno-type	b_i	rank b_i	S^2_{di}	rankS^2_{di}	W^2_i	rank W^2_i	S^2i	rank S^2_i	σ^2_i	rank σ^2_i
A	1.355	2	3.66	5	20.96	6	38.15	8	5.94	6
B	1.113	4	0.7	1	3.24	1	24.6	5	0.24	1
C	0.914	6	6.65	7	20.22	5	20.97	4	5.7	5
D	0.712	7	3.25	4	16.17	3	12	2	4.4	3
E	0.676	8	8.43	8	33.01	9	14.95	3	9.82	9
F	0.992	5	10.47	9	31.65	8	26.7	6	9.38	8
G	1.45*	1	0.76	2	17.37	4	40.66	9	4.79	4
H	0.62	9	5.52	6	28.02	7	11.49	1	8.21	7
I	1.18	3	1.55	3	7.1	2	27.9	7	1.49	2

* indicates slope significantly different from the slope for the overall regression which is 1.00 at $P<0.05$

According to Wricke's stability parameter (W^2i), genotypes with the smallest ecovalance values are considered stable. The (W^2i) was lowest for genotypes B, I and highest for F, E and H. The stability variance (σ^2i) revealed that the genotypes B, I and D had the smallest variance across the environments and were stable, while the genotypes H, F and E had the largest (σ^2i) and were unstable. Best performance in favourable environments belonged to A, G and C but with increase in salt concentration C and H genotypes were appeared better than others (Table 6).

Table 6

Regression coefficient of Verma model for favorable and unfavorable environments

Genotype	Regression coefficient	
	Favorable	Unfavorable
A	1.84	0.73
B	1.33	0.78
C	1.68	0.14
D	0.21	1.16
E	-0.24	1.8
F	0.5	1.6
G	1.61	1.18
H	1.2	0.3
I	0.87	1.32

The number of clusters was determined by the cluster sum of squares in "Elbow criterion" plot. The cluster analysis showed that the genotypes identified based on indices and stability parameters can be divided into three groups (Fig. 3).

DISCUSSION

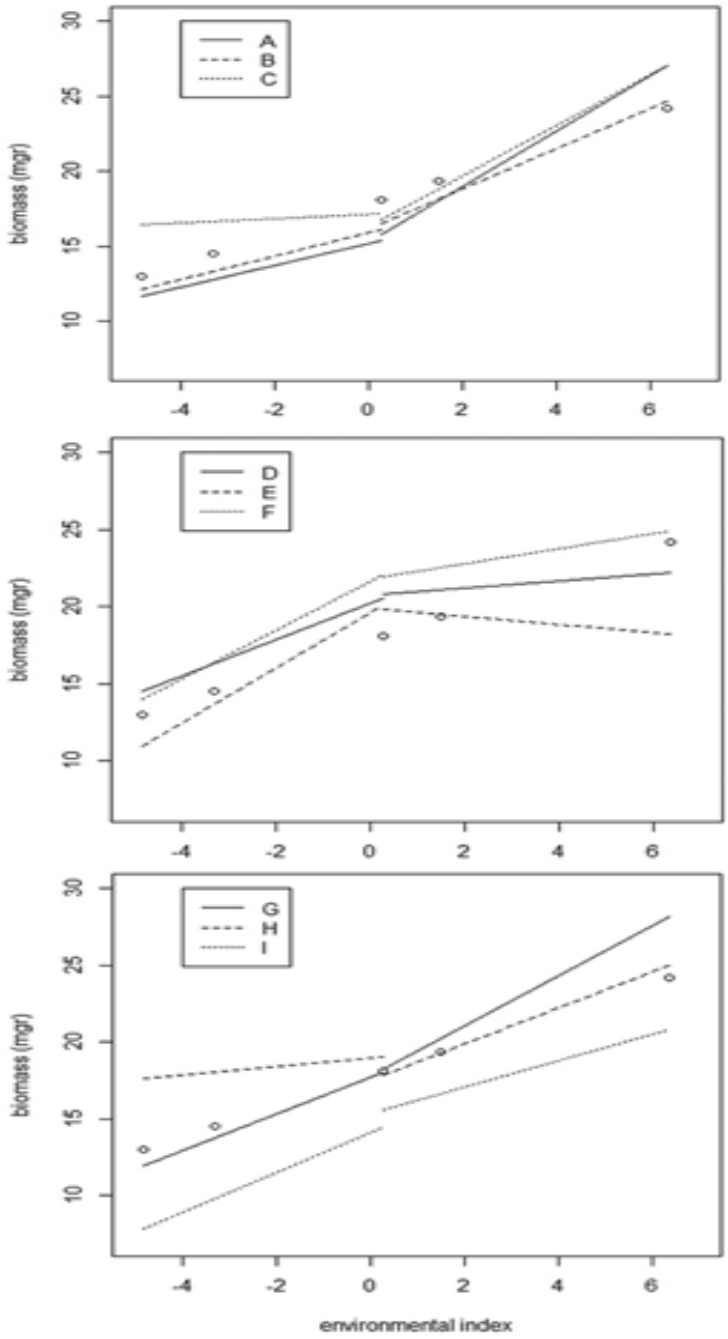

Fig. 1. Dispersion between Yp, Ys and tolerance indices, A: S1 (4.5 ds × m^{-1}) vs. S3 (10.5 ds × m^{-1}), B: S1(4.5 ds × m^{-1}) vs. S5(16.5 ds× m^{-1}).

The result of reduction of dry matter production in the present study are in agreement with Greenway and Munns (1980) who showed this reduction in weights with increasing salinity may be due to limited supply of metabolites to young growing tissues, because metabolic production is significantly perturbed at high salt stress, either due to the low water uptake or toxic effect of NaCl. These results indicate that genetic variation exists among barley genotypes in terms of early seedling growth rate under salt stress condition. According to several reports, a genotype with a highly appropriate response to a certain salinity level cannot necessarily be considered a tolerant genotype.

Instead, a genotype that shows a low yield difference between normal and stress conditions is called tolerant. Fernandez (1992), who studied the yield of genotypes in normal and stress environments, has divided them into four groups: genotypes that have high yield in stress and non-stress environments (group A), genotypes that have a high yield in non-stress environments only (group B), genotypes that have high yield in stress environments (group C) and genotypes that have low yields in stress and non-stress environments (group D).

As an appropriate measure to separate the first group from the other groups, an analysis of the correlation between responses under stress and non-stress conditions as well as quantitative stress tolerance indices, superior indices and consequently genotypes, was used. Generally, indices having high correlations with plant response in stress and non-stress conditions are introduced as the best ones (Ashraf *et al.*, 2015; Ganjeali *et al.*, 2011). In all of the salinity levels correlation between Yp and Ys was very weak, so selection based on genotype response in one of conditions for the anticipation of its performance in other condition will be powerless. As is shown in Fig. 1-A, in spite of SSI high correlation with Yp and Ys there is no determined trend for introducing the genotypes with the best reply in both conditions based on SSI. Nevertheless, according to Fig.1-B , STI can be a suitable index for this proposition.

Plant breeders have a full hand of methods for the analyses of genotype yield adaptability and stability to help in the difficult task of identifying superior cultivars in the presence of genotype × environment interaction (GEI). GEI is important source of variation in any crop and the term stability is sometimes used to characterize a genotype, which shows a relatively constant yield, independent of changing environmental conditions. On the basis of this idea, genotypes with a minimum variance for yield across different environments are considered stable. This idea of stability may be considered as a biological or static concept of stability (Becker and Leon, 1988). This concept of stability is not acceptable to most breeders and agronomists who would prefer an agronomic or dynamic concept of stability; therefore they prefer genotypes with high mean yields and the potential to respond to agronomic inputs or better environment conditions. In the dynamic concept of stability, it is not required that the genotype response to environmental conditions should be equal for all genotypes (Becker and Leon, 1988).

Most of the stability methods indicated that the genotype D was the most phenotypically stable with high mean yield (Table 5). An ideal genotype possesses:

1) high yield performance;

2) low sensitivity to adverse conditions and
3) is capable of responding positively when environmental conditions are improved (Ferreira and Demetrio, 2006). On this fact the ideal genotype has a regression coefficient smaller than 1 for unfavourable environments and greater 1 for favourable environments. Desirable genotypes have concave pattern for regression linear models and C and H genotypes showed such pattern across the levels of salinity (Fig. 2).

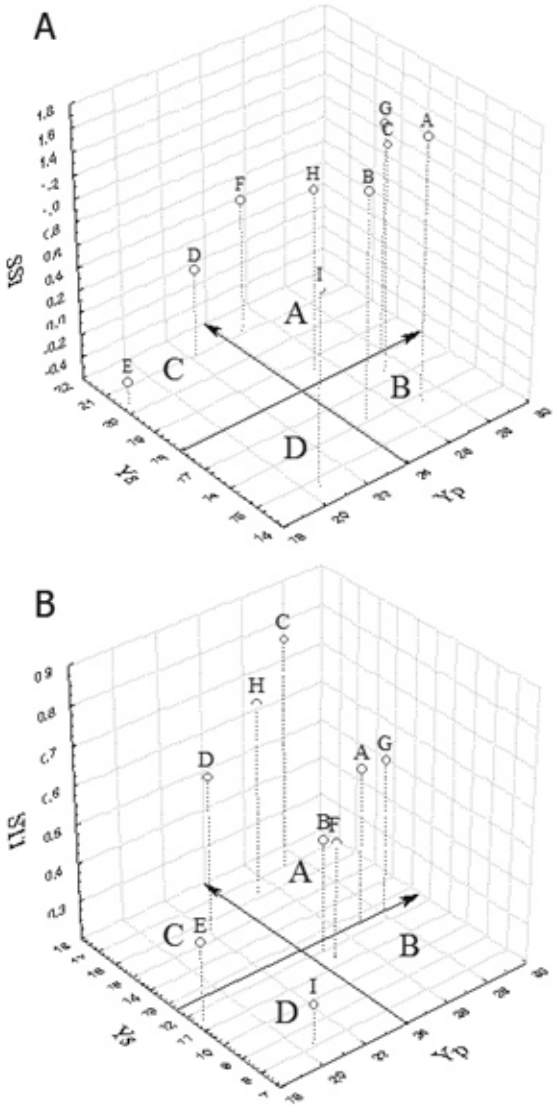

Fig. 2. Performance of barley genotypes across salinity levels based on Verma regression model

In this study, cluster analysis determined that genotypes with high STI, MP and GMP values (Table 7), can be considered the most tolerant and desirable genotypes for both growth conditions. On the other hand it revealed that tolerant genotypes showed the least stability based on mostly of stability parameters. It can be because of variation in their potential for biomass production under different conditions and showed the importance of selection for genotypes performance in both normal and stress environments.

Table 7

Differences of genotype tolerance indices and stability parameters by mean at S5 level of salinity

Genotypes	Property					
	Yp	Ys	TOl	MP	GMP	SSI
A	27	12.67	14.4	19.8	18.5	1.2
B, G, I	24.33	10.77	13.53	17.63	16.27	1.2
C, D, E, F, H	23.46	14.33	9.14	18.96	18.34	0.84
Genotypes	STI	b_i	S^2_{di}	W^2_i	S^2_i	σ^2_i
A	0.6	1.355	3.66	50.96	38.15	5.94
B, G, I	0.47	1.24	1	9.23	31.05	2.17
C, D, E, F, H	0.6	0.78	6.86	25.81	17.22	7.5

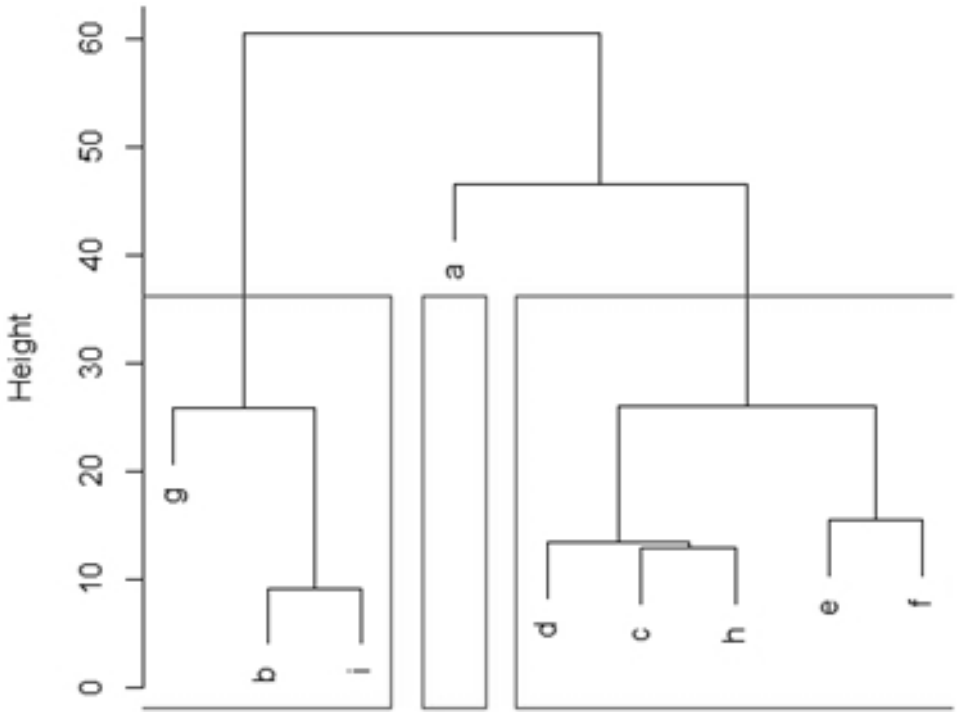

Fig. 3. Cluster analysis of barley genotypes based on tolerance indices and stability parameters

CONCLUSION

The results from this study are very useful for the planning of further barley breeding programs. Salt stress significantly affected the performance of barley genotypes. GMP, MP and STI were more suitable indices for selecting barley genotypes tolerant to salt stress.

The barley selection using these indices can be useful for identifying a cultivar with desirable establishment under both stress and non-stress conditions. Several stability measures that have been used in this study quantified stability of genotypes with respect to yield, stability or both. So both yield and stability should be considered simultaneously to exploit the useful effects of GEI and to refine selection of genotypes. Yet, among all genotypes the WB7910, ESBYTM8910 and MBS8715 showed the best performance in the study.

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EVALUATION OF CARDINAL TEMPERATURES AND THERMAL TIME REQUIREMENT FOR GERMINATION OF *SCROPHULARIA STRIATA* AND *TANACETUM POLYCEPHALUM* (SCHULTZ BIP. SSP. *HETEROPHYLLUM*)

ABSTRACT

Scrophularia striata and *Tanacetum polycephalum* are important medicinal plants in Iran which are rich in essential oils, bitter substances, and sesquiterpene lactones. The present study was conducted to compare four non-linear regression models (segmented, beta, beta modified and Dent-like) to describe the germination rate-temperature relationships of *Scrophularia striata* and *Tanacetum polycephalum* over eight and seven constant temperatures, respectively, to find cardinal temperatures and thermal time requirements to reach different germination percentiles. An iterative optimization method was used to calibrate the models and different statistical indices including RMSE, coefficient of determination (R^2), and AICc were applied to compare their performance. The beta model was found to be the best model to predict germination rate of *Scrophularia striata* at D10, D50 and D90 (R2 = 0.96, R^2 = 0.97, R2 = 0.95; RMSE = 0.005, 0.001 and 0.001, respectively). According to this model outputs, the base, optimum, and the maximum temperatures for germination were estimated as 1.21 ± 0.39, 25.91 ± 0.33 and 46.35 ± 4.12 °C, respectively. Also the segmented model was found to be the best model to predict germination rate of *Tanacetum polycephalum* at D10, D50 and D90 (R^2 = 0.98, R^2 = 0.98, R^2 = 0.98; RMSE = 0.067, 0.59 and 0.56, respectively). According to the model outputs, the base, optimum, and the maximum temperatures for germination were estimated as 0.44±1.15, 26.95±0.75 and 38.33±0.98 °C, respectively. It seems these two medicinal plants need moderate optimum temperature for seed germination.

Keywords: Cardinal temperatures, Seed germination rate, Thermal time, modeling

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INTRODUCTION

Scrophularia genus belongs to Scrophulariaceae family and has five species in Iran (Mozafarian, 1999). *Scrophularia striata* grows in western regions of Iran. It has widely been used as a traditional medicine for treatment of diseases such as eczema, wounds, goiter, ulcers, cancer and fistulae. Both leaves and seeds of *S. striata* contain anti-cancer and cell growth enhancing agents (Ardeshiry lajimi *et al.*, 2010). Scrophulariaceae species have been known to be rich in iridous glycosides, mainly aucubin and catalpol (Park Su *et al.*, 2009). Amiri *et al.* (2011) identified 34 essence compounds in *S. Striata* that contains 90.3% of total essence in this plant. Essential oils of *S. striata* were linalool (18.3%), 6, 10, 14-trimethylpentadecane-2-one (8.4%), dibutyl phthalate (6.9%), and β-damascone (5.9%). *S. striata* extract may stimulate collagen synthesis, faster wound contraction, angiogenesis, vessel dilatation and decrease of inflammation, bleeding and edema (Shoohani *et al.*, 2010).

Tanacetum polycephalum belongs to the compositae family. This plant is an aromatic perennial plant which grows in Caucasia, Iraq, Iran and Turkey (Rechinger, 1986). These members of the daisy family are rich in volatile oils, bitters, and sesquiterpene lactones, which inhibit allergic, inflamatory responses, and are insecticidal.

They are extremely pungent, potent herbs and should be used with caution (Bown, 1995; Keskitalo *et al.*, 2001). Nori-Shargh *et al.* studied the oil of *T. polycephalum* Schultz Bip. ssp. *Heterophyllum* collected from different locations in Iran and found that the main constituents of the oil of the flowers were camphor (59.1%), camphene (14.9%) and 1,8-cineole (10.1%), whereas the leaf oil contained mainly camphor (53.5%), bornyl acetate (12.1%), camphene (10.9%), 1,8-cineole (7.8%) and borneol (6.1%) (Nori-Shargh, 1999).

S. striata and *T. polycephalum* are endangered medicinal plants because of excessive harvest from natural habitats for traditional use.

In order to determine the best planting date for crops, it is necessary to find the base (T_b), optimum (T_o) and maximum temperatures (T_c) for seed germination which are known as cardinal temperatures (Bewley and Black, 1994). Cardinal temperatures are determined for many of agronomic crops while for most weed species and medicinal plants, they should be determined. Modeling of seed germination is known as a good approach in determination of cardinal temperatures, but it should be noted that due to unpredictable biological phenomena, they have some limitations. Usually a linear increase in germination rate is associated with increasing temperature from a base temperature (T_b) up to an optimum, then it shows linear reduction tread to a ceiling temperature (Garcia-Huidobro *et al.*, 1982; Steinmaus *et al.*, 2000; Bradford, 1990; 1995; 2002; Rowse and Finch-Savage, 2003). To perform seed germination modelling, two main concepts have widely been used by researchers:_Empirical model and Mechanical models.

Empirical models can do a great job in various levels of the empiricism of matching individual data of germination overtime, while such models may need more empirical variables (Brown and Mayer, 1988). The empirical method may be useful for specific jobs, but it is difficult to elucidate the biological significance for appraisaling model parameters (Bradford, 1990).

Mechanical models are based on experimental quantifying of environmental effects on seed germination and seedling emergence. This approach has the highest chance of success in the long run (Bradford, 1990; Forcella *et al.*, 2000). It has been shown that mechanical threshold models for seed germination and seedling emergence have delivered some success (Forcella, 1993; Benech-Arnold and Sánchez, 1995; Allen *et al.*, 2000; Roman *et al.*, 2000; Bradford, 2002; Rowse and Finch-Savage, 2003). Kamkar *et al.* (2005, 2008) reported that segmented and logistic models could be used for determination of cardinal temperatures in three millet varieties and seedling emergence of wheat cultivar "Tajan". Other functions such as power (Stapper and Lilley, 2001), the beta (Yin *et al.*, 1997), the sigmoid, the exponential (Angus *et al.*, 1981) and intersected functions (Kamkar *et al.*, 2005, 2008) are widely used to describe crop responses to temperature.

These regression models estimate cardinal temperatures. In dent-like model at a lower temperature than optimum, linear relationships is exited between temperature and germination rate, while this relationship has also remained linear at higher temperatures than optimum but in reduction trend. In the segmented, with increasing the temperature, germination rate increases linearly till reach to optimum temperature, after this point a constant trend is produced. According to the literature, there is not any information about germination of these two medicinal plants and this study appears to be the first report about cardinal temperatures of these two species germination.

The objective of this study was to test various model responses and also to test whether beta and beta modified models can work better than segmented and dent-like in estimation of cardinal temperatures for seed germination in *S. striata* and *T. polycephalum*.

MATERIALS AND METHODS

Cardinal temperatures determination

An experiment was performed to determine the cardinal temperatures of *S. striata* and *T. polycephalum*. The experiment was conducted using germinators with controlled environments in the Seed Laboratory, University of Tehran, Karaj, Iran. Four replications of 50 seeds were germinated in 9 cm diameter Petri dishes on two layers of Whatman No. 1 (9 cm diameter) filter paper containing 5 ml distilled water. The germination response was evaluated at eight constant temperatures of 5, 10, 15, 20, 25, 30, 35 and 40˚C for *S. striata* and seven constant tem-

peratures of 5, 10, 15, 20, 25, 30 and 35 for *T. polycephalum*.
A seed was considered as germinated when its protruded radicle elongated at least 2 mm. The germinated seeds were counted every 24h under different temperatures. The time from the start of the imbibition to the last germination was considered the total time to maximum germination. The cumulative germination percentage was plotted against time (h). From this curve, the time to 50% germination (D50) was determined by fitting a logistic model to cumulative germination percentage (G) against time (t, h) as described by equation 1:

$$G = \frac{G_x}{1 + exp[a \times (t - b)]}$$

where: G_x is the maximum germination percentage, b is the time for 50% germination. The times for 10%, 50% and 90% germination were also determined by interpolation and are designated D10, D50 and D90, respectively (Marshall and Squire, 1996; Shafii and Price, 2001; Soltani, 2007).
The reciprocal of the time taken for a given fraction of the seed population to germinate was considered to be the germination rate (GR).
To quantify the response of the germination rate of temperature and cardinal temperatures for germination, the following equation was used:

$$GR = \frac{f(T)}{f_0}$$

where: *f(T)* is a T function (reduction factor) that ranges between 0 at the base and maximum temperatures and 1 at the optimal temperature(s), and $1/f_o$ is the inherent maximum rate of germination at the optimal temperature estimated via an iterative optimization method. Therefore, the minimum number of hours for germination at the optimal temperature was calculted (Soltani *et al.* 2006). The *GR* also shows the germination rate of a given percentile. The Sigma Plot software was used to calibrate the models (beta, beta modified, segmented and dent-like) via an iterative optimization method (Table 1). To determine the best estimates of the parameters (lower biases of the intercept from 0 and the slope from 1 are criteria for increased reliability), (*RMSE*; Equation 3), the coefficient of determination (R^2; Equation 4), and the intercept and slope of the regression equation of predicted vs. observed germination rate were used. MAE was used because it avoids compensation between probable under- and over-prediction as follows:

$$RAMSE = \sqrt{\left(\frac{1}{n}\right) \times \sum \left(Y_{obs} - Y_{pred}\right)^2}$$

where: Y_{obs}: observed value, Y_{pred}: predicted value, n: number of samples (Timmermans, 2007).
and

$$R^2 = \frac{SSR}{SST}$$

where: D_i is the difference between measured and calculated values, *SSR* is the sum of squares (SS) of regression ($\sum_{i=1}^{n}(\hat{L} - \bar{L})$) and *SST* is the total *SS* ($\sum_{i=1}^{n}(L_1 - \bar{L})$). Y_i is the observed value and $\hat{L}$ is the correspondent estimated value. The parameters estimated by non-linear models were exposed to descriptive statistical analysis for the pooled datasets, after which the best estimated values were used to calculate the thermal time needed for each germination percentile. Lower *RMSE* and R^2 near to 1 show better model estimation.

To determine the best model in the estimation of cardinal temperature, Akaike Information Criterion (AIC) is used. This index explain the amount of reduction RSS, value of reduction from a degree of freedom of error and model complexity (Burnham and Anderson, 2002).

$$AIC = n \times Ln\left(\frac{RSS}{n}\right) + 2 \times k$$

where: *RSS* is Residual Sum of Square, *n* – number of observation and *k* is a number of model parameters.

It is possible to use corrected *AIC* (*AICc*) index instead of using *AIC*. This index is used to determine most accurate model (Butler and King, 2004; O'Meara *et al.*, 2006).

$$AICc = n \times Ln\left(\frac{RSS}{n}\right) + 2 \times k + \left(\frac{2 \times k \times (k+1)}{n-k-1}\right)$$

The model that produces a more accurate estimation is the one with the lower AICc value. Although the best model is the one that produces lower *AICc*, but there is a method that by using is, we are able to explain, rank and fit different models. This method is perform with calculation of $\underline{\Delta}i$.

$$\Delta_i = AICc - minAICc$$

where: *minAICc* is the minimum value of calculating *AICc* among all models, and it belongs to the model that best fitted. If ΔI<10, then it means that there is no significant difference between models and model with higher *AICc* could also be fitted well. While Δ*I*>10, then model with higher *AICc*, is not suitable and could not be fitted well.

Thermal time determination

The daily thermal time (DTT) was calculated as:

$$DTT = (T_{o1} - T_b) \times f(T)$$

where *f(T)* is the *T* function, T_{o1} is the lower optimum *T*, and T_b is the base *T*. The first components of daily thermal time are the constant and non-optimal temperatures that affect the daily thermal time through *f(T)*.

Table 1

Models that were fitted to germination rate vs. different constant temperatures

Function	Formula	Reference
Beta, five parameters	$f(T)=\left(\frac{(T-T_b)}{(T_o-T_b)}\right)\times\left(\frac{(T_c-T)}{(T_c-T_o)}\right)^{\left(\frac{(T_o-T_b)}{(T_c-T_b)}\right)^c}$	Yin *et al*., 1995
Beta, four parameter	$f(T)=\left(\frac{(T_c-T)}{(T_c-T_o)}\right)\times\left(\frac{(T-T_b)}{(T_o-T_b)}\right)^{\left(\frac{(T_o-T_b)}{(T_c-T_b)}\right)^c}$	Yan and Hunt, 1999
Dent-like	$f(T)=\left(\frac{(T-T_b)}{(T_{o1}-T_b)}\right)\ if\ T_b<T<T_{o1}$ $f(T)=\left(\frac{(T_c-T)}{(T_c-T_{o2})}\right)\ if\ T_{o2}<T<T_c$ $f(T)=1\ if\ T_{o1}\le T\le T_{o2}$ $f(T)=0\ if\ T\le T_b\ or\ T_c\le T$	Piper *et al*., 1996
Segmented	$f(T)=\left(\frac{(T-T_b)}{(T_o-T_b)}\right)\ if\ T_b<T<T_{o1}$ $f(T)=1-\left(\frac{(T-T_o)}{(T_c-T_o)}\right)\ if\ T_o\le T<T_c$ $f(T)=0\ if\ T\le T_b\ or\ T_c\le T$	Mwale *et al*., 1994

Beta, segmented and dent-like , where T is the temperature, T_b the base temperature, T_o the optimum temperature, T_{o1} the lower optimum temperature (for 3-piece segmented function), *To2* the upper optimum temperature (for 3-piece segmented function), Tc the maximum temperature, c is the shape parameter for the beta function which determines the curvature of the function and d is the parameter of the beta modified function which indicates the sensitivity of the germination rate to temperature.

RESULTS AND DISCUSSION

In this study, *AICc* and R^2 were the main indices for selection of the best model for evaluation of cardinal temperatures of the two species. Estimated parameters for the dent-like, segmented and beta (4 and 5 parameter) models for different seed germination percentiles of *S. striata* and *T. polycephalum* seed is shown in Table 2 and Table 3 respectively. Also predicted and observed seed germination rate of *S. striata* and *T. polycephalum* for different germination percentiles (D10, D50 and D90) using following models:

- beta (a),
- beta modified (b),
- segmented (c)
- dent-like (d)

The models are shown in Fig. 1 and Fig. 4, respectively. Beta five-parameter model was shown to be more successful to evaluate cardinal temperatures of *S. striata* than other models.

According to this model, calculated *AICc* indexes were equal:

- -308.02, for D10
- -361.53 for D50
- -371.88 , for D90,

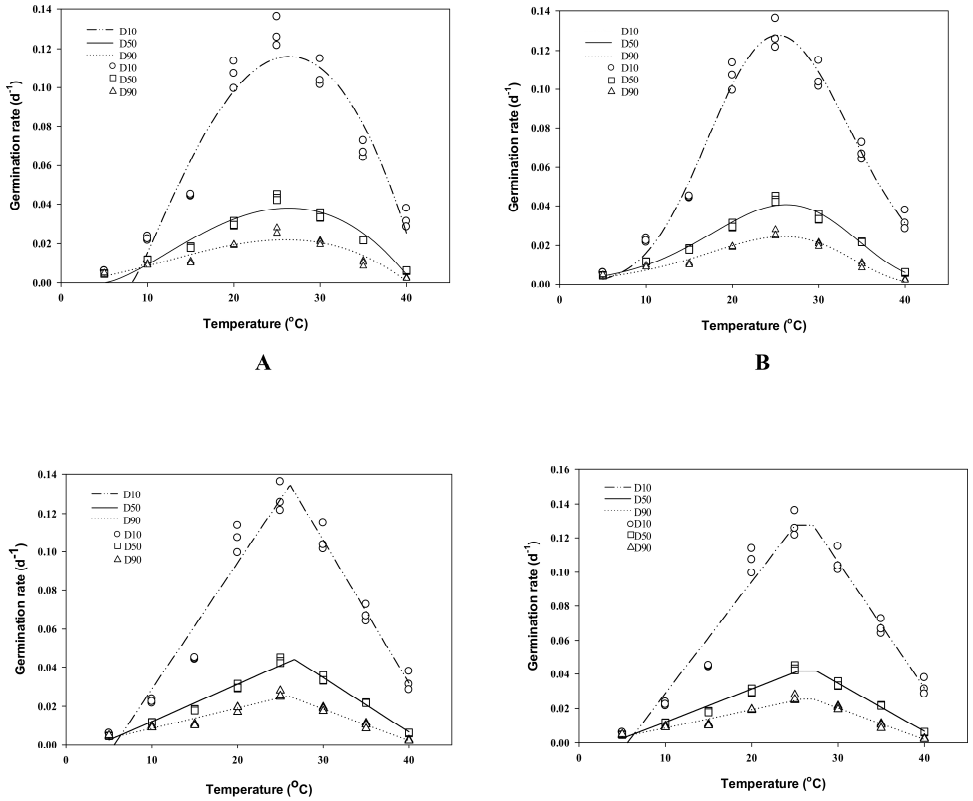

Fig. 1. Predicted (lines) versus observed (symbols) seed germination rate of *S. striata* at different constant temperatures for different germination percentiles (D10, D50 and D90) using beta (a), beta modified (b), segmented (c) and dent-like (d) models

Moreover, this model was most reliable for D10, D50 and D90, due to the higher determination coefficient between observed and predicted values (R^2 = 0.96, 0.97 and 0.95 for pooled data). According to beta five- parameters model cardinal temperatures were T_b (4.30 ± 1.29, 3.25 ± 0.79, 1.21 ± 0.39), To (25.28 ± 0.54, 26.23 ± 0.36, 25.91 ± 0.33) and T_c (40.21 ± 0.33, 42.32 ± 0.16, 46.35 ± 4.12) for D10, D50 and D90 respectively (Table 2). Furthermore beta modified model had the lowest accuracy in predicting the cardinal temperatures of *S. striata* because of the lowest R^2 and the highest AICc than other models. In beta modified model,

AICc was -234.24, -286.29 and -345.15 for D10, D50 and D90, respectively (Table 2). For *T. polycephalum* segmented model had the highest accuracy in predicting cardinal temperatures. According to this model, calculated *AICc* index was -60.52, -80.56 and -87.04 for D10, D50 and D90, respectively. In addition, this model was most reliable for D10, D50 and D90, because of the higher determination coefficient between observed and predicted values (R2 = 0.98, 0.98 and 0.98 for pooled data) (Table 3). According to segmented models cardinal temperatures for *T. polycephalum* were T_b (2.55 ± 0.95, 1.70 ± 0.83, 0.44 ± 1.15), To (27.36 ± 0.81, 23.56 ± 0.61, 26.95 ± 0.75) and T_c (38.74 ± 1.24, 39.42 ± 1.02, 38.33 ± 0.98) for D10, D50 and D90, respectively (Table 3).

Table 2

Estimated parameters for the SEGMENTED, BETA, BETA MODIFIED and DENT-LIKE models for different germination percentiles of *S. striata* seed

Parameter[1]	Segmented			Beta		
	D10	D50	D90	D10	D50	D90
T_b	5.60 ± 0.67	3.70 ± 0.54	1.78 ± 0.94	4.30 ± 1.29	3.25 ± 0.79	1.21 ± 0.39
T_o	26.14 ± 0.69	26.64 ± 0.41	25.89 ± 0.63	25.28 ± 0.54	26.23 ± 0.36	25.91 ± 0.33
T_c	44.29 ± 1.16	42.42 ± 0.53	41.33 ± 0.68	40.21 ± 0.33	42.32 ± 0.16	46.35 ± 4.12
f_o	7.42 ± 0.26	22.70 ± 0.47	39.62 ± 1.22	7.83 ± 0.27	24.59 ± 0.54	40.87 ± 1.65
c	-	-	-	4.83 ± 0.21	2.12 ± 0.17	1.07 ± 0.49
R^2	0.94	0.97	0.95	0.96	0.97	0.95
RMSE	0.009	0.001	0.001	0.005	0.001	0.001
AIC	-291.70	-367.31	-375.79	-312.02	-366.53	-376.88
AICc	-283.70	-359.31	-367.79	-308.02	-361.53	-371.88
Δ_i	24.32	2.21	4.08	0	0	0
Parameter[1]	Beta modified			Dent-like		
	D10	D50	D90	D10	D50	D90
T_b	8.17 ± 1.31	5.00 ± 2.50	1.51 ± 0.86	5.60 ± 0.70	3.70 ± 0.56	1.82 ± 0.94
T_o	26.43 ± 0.71	26.37 ± 0.69	25.99 ± 0.93	-	-	-
T_c	41.72 ± 0.60	40.76 ± 0.41	40.20 ± 0.48	44.29 ± 1.21	42.42 ± 0.54	41.00 ± 0.59
T_{o1}	-	-	-	25.05 ± 0.83	25.39 ± 0.92	25.83 ± 0.54
T_{o2}	-	-	-	27.11 ± 0.35	27.49 ± 0.09	27.13 ± 0.02
f_o	8.60 ± 0.31	26.34 ± 0.83	45.69 ± 2.04	7.84 ± 0.78	24.07 ± 0.30	39.28 ± 0.78
R^2	0.91	0.92	0.88	0.94	0.97	0.95
RMSE	0.011	0.003	0.002	0.009	0.001	0.001
AIC	-242.24	-294.29	-353.15	-289.70	-365.31	-374.21
AICc	-234.24	-286.29	-345.15	-285.70	-361.31	-370.28
Δ_i	73.78	75.23	76.72	22.32	0.21	1.59

T_b , T_o ,T_c ,T_{o1} , T_{o2} ,f_o and care base temperature, optimum temperature, maximum temperature, lower limit of optimum temperature, upper limit of optimum temperature, minimum time to reach a given percentile, parameter of beta function, coefficient of regression, respectively

The base and the maximum temperatures for different percentiles did not show any significant difference for all tested models for *T. polycephalum*. The beta-modified and dent-like models, were also reliable for D10 and D50 (Table 3), because R^2 was high for both models. According to the segmented model for percentiles of D10, D50 and D90, the basic temperature varied be-

tween 2.55 ± 0.95 and 0.44 ± 1.15°C and estimated ceiling temperatures for D50, D90 was 39.42±1.02 and 38.33 ± 0.98, respectively (Table 2).

Table 3

Estimated parameters for the SEGMENTED, BETA, BETA MODIFIED and DENT-LIKE models for different germination percentiles of *T. polycephalum* seeds

Parameter[1]	Segmented			Beta		
	D10	D50	D90	D10	D50	D90
T_b	2.55 ± 0.95	1.70 ± 0.83	0.44 ± 1.15	2.02 ± 0.18	1.46 ± 0.64	1.01 ± 10.18
T_o	27.36 ± 0.81	23.56 ± 0.61	26.95 ± 0.75	26.06 ± 1.25	22.59 ± 0.78	25.09 ± 1.38
T_c	38.74 ± 1.24	39.42 ± 1.02	38.33 ± 0.98	38.05 ± 5.58	38.34 ± 3.03	35.97 ± 1.63
f_o	7.13 ± 0.31	18.00 ± 0.10	29.30 ± 1.09	7.95 ± 0.56	20.85 ± 0.87	34.27 ± 2.02
c				4.71 ± 9.16	1.98 ± 1.08	1.74 ± 1.56
R^2	0.98	0.98	0.98	0.97	0.97	0.94
RMSE	0.67	0.59	0.56	0.02	0.004	0.004
AIC	-73.52	-93.56	-100.04	-69.22	-92.28	-94.90
AICc	-60.52	-80.56	-87.04	-39.22	-62.28	-64.90
Δi	0	0	0	21.3	18.28	22.14
Parameter[1]	Beta modified			Dent-like		
	D10	D50	D90	D10	D50	D90
T_b	2.13 ± 0.19	0.13 ± 1.73	-1.85 ± 1.79	2.55 ± 1.10	1.42 ± 0.75	-1.04 ± 1.31
T_o	26.30 ± 0.82	22.78 ± 0.60	24.86 ± 0.87			
T_c	36.85 ± 0.64	37.21 ± 0.55	36.93 ± 0.64	38.74 ± 1.43	38.94 ± 1.26	38.33 ± 1.08
T_{o1}				25.88 ± 0.44	18.21 ± 1.19	23.62 ± 2.02
T_{o2}				28.03 ± 0.80	26.57 ± 1.25	28.00 ± 1.05
f_o	8.05 ± 0.43	21.10 ± 0.67	33.67 ± 1.50	7.58 ± 0.09	21.27 ± 0.78	32.25 ± 1.85
R^2	0.95	0.97	0.95	0.95	0.97	0.96
RMSE	0.02	0.005	0.004	0.01	0.004	0.003
AIC	-71.22	-92.69	-96.60	-71.52	-94.03	-99.09
AICc	-58.22	-79.69	-83.60	-41.52	-64.03	-69.09
Δi	02.30	0.87	3.44	19.00	16.53	17.95

T_b , T_o ,T_c ,T_{o1} , T_{o2} ,f_o and care base temperature, optimum temperature, maximum temperature, lower limit of optimum temperature, upper limit of optimum temperature, minimum time to reach a given percentile, parameter of beta function, coefficient of regression, respectively

Dent-like model produced the lowest RMSE for D10, D50 and D90 (0.01, 0.004 and 0.003, respectively) compared to other models for *T. polycephalum* (Table 3). In this study, the germination rate was very sensitive to temperature, in order that it was slow at low temperatures. The germination rate is maximum at optimum temperature and by increasing and decreasing in temperature, germination rate decreases. Decreasing of germination rate at low temperatures is related to decrease imbibition rate of seed (Bewley and Black, 1994). Khan *et al* (2001) investigated the effects of different temperature regimes on germination of *Kochia scoparia* and reported that the temperature had a significant effect on germination, and germination rate was higher at higher temperatures. Several reports indicate an increasing effect of temperature on the speed of germination up to a certain point (Hardegree and Winstral, 2006; Bannayan *et al.*, 2006).

Other studies suggest that the typical germination rate increases linearly with increasing temperature in a suitable range of temperature, but at higher tempera-

tures it decreases (Mwale *et al.*, 1999). Adam *et al.* (2007) stated that the germination response to temperature could be different depending on the species or populations within a species. Decrease of germination rate at low temperatures is related to decreased seed imbibition rate (Begley and Black, 1994). Tabrizi *et al*. (2007) by Evaluation of various models on germination of two agricultural and natural populations of Thyme (*Thymus Transcaspicus*) reported that beta five-parameter model has the most reliable of cardinal temperatures for germination on natural population of this species. In addition, among different nonlinear regression models (Dent-like, segmented and beta), segmented model was found to be the best model to predict germination rate of opium poppy (Kamkar *et al.*, 2012). In segmented model, relative changes in development rate is plotted separately for temperatures lower and more than optimum temperature. The optimum temperature calculated from the intersection of two regression lines and base temperature and maximum are intercept of the regression line at lower and more temperatures than the optimum temperature, respectively (Phartyal *et al.*, 2003).

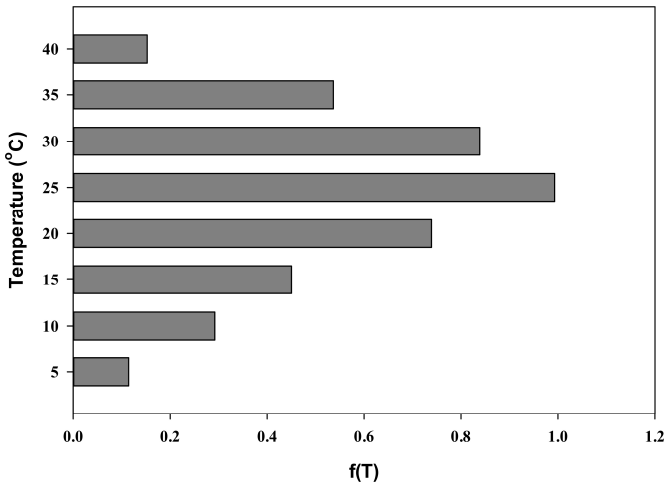

Fig. 2. *f(t)* Values for different constant temperatures based on beta model

Calculated *f(t)* for constant temperatures used in this research based on the beta model for *S. striata* and segmented model for *T. polycephalum* are illustrated in Fig 2 and Fig 5 respectively. They show an increase trend to 25°C then starts to decrease for the two species. This suggests that the optimum temperature for two species is around 25°C .Using the estimated parameters of the segmented model, each germination percentile will be achieved when $DTT = TT$, or $f(T) = f_o$, or $f(T)/f_o = 1$. It is clear that temperatures closer to optimum temperature have a small reducing effect on germination rate.

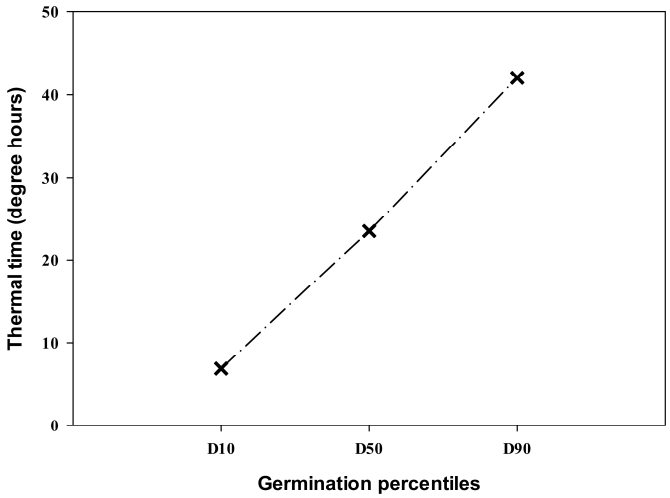

Fig. 3. Thermal time (degree-hour) required for different germination percentiles in based on pooled data, when $T = T_o$

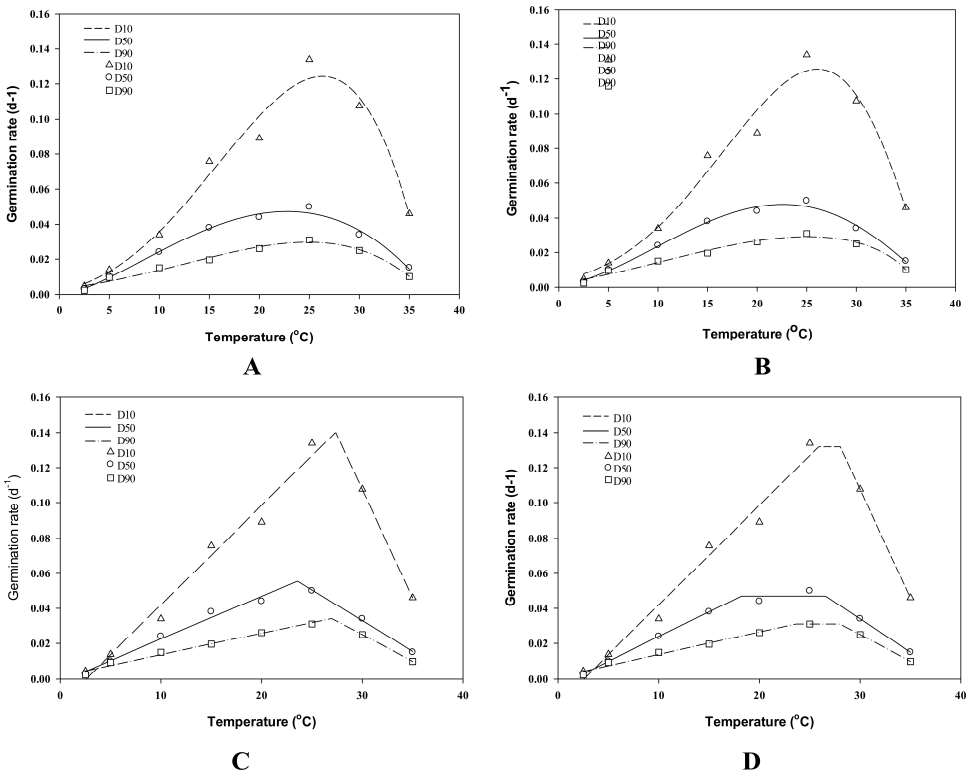

Fig. 4. Predicted (lines) vs. observed (symbols) germination rate of *T. polycephalum* seeds at different constant temperatures for different germination percentiles (D10, D50 and D90) using beta (a), beta modified (b), segmented (c) and dent-like (d) models

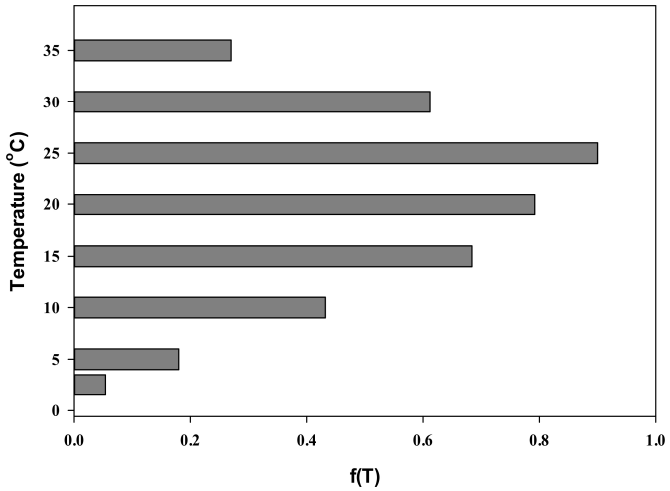

Fig 5. *f(t)* Values for different constant temperatures based on the beta model

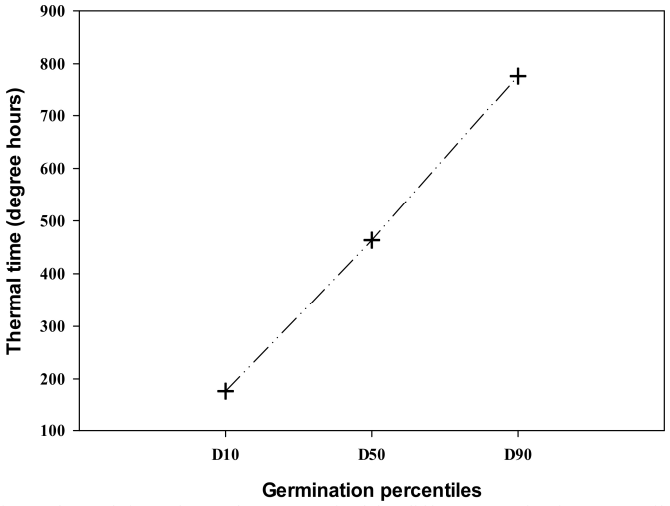

Fig 6. Thermal time (degree-hours) required for different germination percentiles in based on pooled data, when $T=T_o$

The calculated thermal times for each germination percentile based on pooled data are represented in Fig. 3 and Fig 6 for *S. striata* and *T. polycephalum* respectively. Thermal time required for 10, 50 and 95% germination in *S. striata* is 7, 23 and 43 degree-days respectively. Also thermal time required for 10, 50 and 95% germination in *T. polycephalum* is 170, 460 and 780 degree-hours respectively. Kamkar et al (2012) reported that the thermal time required to reach 50 and 95% germination in opium poppy was 57.27 and 87.55 degree-days, respectively. The thermal time requirement for a developmental process (like germination), offers a measure of physiological time required to complete the process. In addition, thermal time is the number of degree days required for

a developmental process based on a set of physiological temperatures during the process and expression of time in thermal units. It eliminates the time dependence of biological process because of temperature change (Trudgill *et al.*, 2005). Thermal time required for each developmental stage is calculated by inversion the slope of the regression function of development rate versus temperatures below the optimum temperature (Thornley, 1987).

Our results confirmed the certainty of the estimated parameters and the reliability of the beta for *S. striata* and segmented model for *T. polycephalum*. In other words, the regression between the degree day sums and the mean temperatures for this experiment confirmed independently between the degree day sums and the temperatures of traits. This independency has fully explained by Bonhomme, (2000) for using degree day's unit in such experiments. This study suggests that the bilinear-shape response model of germination rate of temperature can be used to estimate the cardinal temperatures of *T. polycephalum*. In this model, the germination rate is regressed separately against temperature for two extreme of temperatures (below and above optimum temperature). Base temperature and maximum temperature are the intercepts of each regression line (Covell *et al.*, 1986; Phartyal *et al.*, 2003). The results of the present study confirmed that in the absence of other limiting factors (e.g., light, water), seed germination of *S. striata* and *T. polycephalum* is highly influenced by temperature. In addition, our results indicate that the germination rate of *S. striata* based on the beta model and *T. polycephalum* based on the segmented model exhibite sharply defined cardinal temperatures.

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Plant Breeding and Seed Science publishes articles reporting the results of original research, critical reviews and interpretive articles in all field of plant breeding and seed production. Submissions to PB&SS must not be published in or submitted for publication elsewhere. All manuscripts will be referred by two reviewers and published in order of the acceptance. Authors are anonymous from the reviewers and reviewers are from authors. ***Manuscript***, including tables and figures, should not exceed ***20 A4 pages*** (there is about 1600 characters per 1 page including points, commas and spaces). Text should be **double spaced**, typed on one side only with ***4 cm left margins*** (2,54 cm right, bottom and top margins). Two legible copies and manuscript on disk should be submitted.

Arrangement of the manuscript

The title page of manuscript should include the following: the title, the name(s) of the author(s), the name(s) and the address(es) of the institution(s) where work was done.

Abstract — not exceeding 250 words and **Key words (maximum seven)** arranged alphabetically.

Main text should be normally divided into: introduction, material and methods, results, discussion and conclusions.

Acknowledgements — if any (including grants etc.) must be on a separate page

The reference list must be in alphabetical order by the first author. References should include: the author name(s), the year of publication, complete title, abbreviated title of journal, volume number, first and last page. Book references should include, names of all authors, the editor, year, title, publisher and place of publication.

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Each table should be typed on a separate page and their position in the text should be suggested in the margin. Tables should be numbered using Arabic numerals followed by the title. They should be referred to in the text by their number. Vertical rules should not be used.

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The International System of Units (SI) and abbreviation is required. Non standard abbreviations should be explained when they first appear in the text. All Latin names should be written in italics. References in the text should contain the name(s) of the author(s) and the year of publication.

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