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Original scientific paper

ULTRASOUND INFLUENCE ON COLEOPTILE LENGTH AT *POACEAE* SEEDLINGS AS VALUABLE CRITERIA IN PREBREEDING AND BREEDING PROCESSES

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The study includes research on the effect of ultrasound on the ability of seed germination and coleoptile development of cereal landraces (fam. *Poaceae*): oat (*Avena sativa* L.) brodski, rye (*Secale cereale* L.)

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rakotinska, *Triticale* svetinikolsko and soft wheat (*Triticum aestivum* L.) govrlevska. The experiment with ultrasound was carried out at frequency of 30-40 kHz on the thermostatic ultrasonic bath. Seeds were sonicated at a constant temperature (25ºC) for 15 min. Ultrasound effect is reflected in the length of the coleoptyl and mesocotyl, although in a good deal is genetically predetermined. Coleoptile length was the longest in *Triticale* (3.3-5.5 cm) and approximately the same lengths are evident in rye (3.0-6.1 cm). Concerning the mesocotyl the longest one (5.525 cm ± 0.697) is recorded at rye, even significantly longer than control of oat and *Triticale*. Longer coleoptile of sonicated seeds indicates faster seedling development, good water supply and rapid development and emergence of first leaves. Correlation coefficient showed very high (0.821) and high R^2 (67,472%) dependence among variables both, in length of coleoptiles (as dependent variable) and mesocotyl (as independent) with low standard error (0.225). With the simple use of ultrasound the germination period could be shorten, water supply more efficient, the sowing periods will be shorten and good yields even under conditions of climate change with increased temperatures could be achieved

Key words: coleoptile length, mesocotyl, seedlings, ultrasound.

INTRODUCTION

Agro biodiversity is not only historical heritage, because a necessity to preserve the genetic material of traditional varieties and landraces is growing. Plant diversity provides valuable traits needed for facing future challenges, such as increased food and feed demands adaptation to climatic changes and biotic stress. Utilization of plant diversity, preserved in gene banks is important task for breeders. Nowadays, one of priorities when climate change is evident, visible and very vulnerable is breeding for abiotic stress tolerance (KAVAR *et al.,* 2008; BABIĆ *et al.,* 2011). Very large and very important feature for adaptation to dry climate conditions is the length of seedlings coleoptiles (STANEVICIUS *et al.*, 2008; SIMEONOVSKA *et al.*, 2010). To obtain the longer coleoptiles ultrasound has been used as a possible technology to achieve greater value of this trait. Ultrasonic waves activate the enzyme, accelerated the starch metabolism (alpha-amylase activity) (YALDAGARD *et al.*, 2007; 2008) and the overall biochemical processes that occurs during seed germination (KRATOVALIEVA *et al*.,2012). This technology has been increasingly used to obtain higher percentage of germination (SAWERS *et al.*, 2002), faster growth, development of healthy seedlings and potentially a good yield. The development of longer coleoptile protects the seedling inside and roll-curved first leaves, allows larger amount of absorbed water and essential materials for seedling growth and development. Simultaneously, a significant finding is the importance of mesocotyl length that has not been studied in relation to the length of coleoptile. From etiolated mesocotyl cells are differentiated in hypocotyl and further develop root system (nodal roots, radical and lateral seminal roots). Priming of seeds with ultrasound was the initial step in testing of possible influence on the length of coleoptiles and mesocotyl.

Landraces have developed adaptation mechanisms to local environment over many years of growing (DE CASTRO and CAPOTE, 2007; ANDJELKOVIC *et al.,* 2011). With global climate changes it becomes necessary to chose among them the genetic material suitable for satisfactory yield in these conditions.

MATERIALS AND METHODS

Plant material

Research was conducted on four landraces of small grain cereals, well-known and locally adapted for more than 30 years in different regions of Macedonia. Collected during national expedition code SVEBRO_2010/2011, landrace material was kept at long-term (-18°C) storage with specific characteristics for each of them according to FAO / IPGRI (1993) descriptors; oat (*Avena sativa* L.) MKD03047 brodski, rye (*Secale cereale* L.) MKD03131 rakotinski, *Triticale* (x *Triticosecale*) MKD03428 svetinikolski and soft wheat (*Tr.aestivum* L.) MKD03080 govrlevska.

Germination test

Seeds were germinated in 100 mm Petri dishes (100 seeds per dish) on a filter paper layer (120g) moistened with distilled water. The samples were placed in a growth chamber at 20^{o}C and incubated at the low humidity (70-75%) for 8 days, followed by 7 days at 25^{o}C. The constant darkness and mentioned relative humidity was maintained throughout the experiment. Taken procedure was replicated four times for all accessions. Recording of the results was done according the national standards without pre-refrigeration, with a 12 hour day/night alternation: 20/17 for oat, 20/15 for rye, 20/15 for triticale and 20/17 for soft wheat. The length of coleoptile and mesocotyl were measured after 15 days.

Priming of seeds with ultrasound

5 g seeds of each landrace were dispersed in 50 ml of tap water (135 ppm TDS, Liquatec, Fullerton USA) in 50 ml laboratory glass beaker. The experiment was performed at a power output of 300 W. The solution was processed at a constant temperature of 25ºC with the sonication for 15 min.

Statistical analysis

The results were processed and tested using STATGRAPHICS Centurion XVI (StatPoint Technologies, Inc., USA). The least significant difference (LSD) and correlation coefficient were determined at $p \leq 0.05$. The analysis of variance was performed for the coleoptile and mesocotyl length. Coleoptile stability was estimated using exponential model: Y = exp (a+b*X). Evaluation of the slope of linear regression on the coleoptile length (dependent variable) and mesocotyl length (independent variable) was calculated for both variables.

RESULTS AND DISCUSSION

The coleoptile length was the longest in *Triticale* and ranges from 3.3 to 5.5 cm in ultrasound (US) treated seeds, respectively from 3.1 to 4.0 cm in control (Table 1). Approximately the same lengths of coleoptiles were measured in rye which ranks within 3.0-6.1 cm in ultrasonicated seeds and 2.1-3.1 cm in control. Shorter coleoptiles developed seedlings in soft wheat (3.3-4.4 cm) US treated compared to non-treated (3.0-4.0 cm), while the shortest were those in oat (2.0-3.8 cm) and from 0.8 to 2.0cm in control. According to grouping using StatPoint programme, averagely longest coleoptile has *Triticale* 4.460 cm, only slightly shorter ray 4.395 cm while significantly shorter was in soft wheat 3.792 cm and 2.965cm as the shortest in oats.

Table 1. Influence of ultrasound on the length of coleoptile and mesocotyl (mean ± σ)

Crop	Coleoptile length (cm)		Mesocotyl length (cm)	
	USPS (x ± σ)	CS (x ± σ)	TS (x ± σ)	CS (x ± σ)
Avena sativa L.	$2.965^{a} \pm 0.429$	$1.352^{b} \pm 0.330$	$3.662^{a} \pm 0.411$	$2.202^{b} \pm 0.312$
Secale cereale L.	$4.395^{a} \pm 0.639$	$2.662^{b} \pm 0.207$	$5.525^{a} \pm 0.697$	$4.530^{b} \pm 1.110$
Triticale (xTriticosecale)	$4.460^{a} \pm 0.596$	$3.367^{b} \pm 0.293$	$5.180^{a} \pm 0.584$	$4.785^{b} \pm 0.862$
Triticum aestivum L.	$3.792^{a} \pm 0.264$	$3.585^{b} \pm 0.192$	$4.822^{a} \pm 0.722$	$4.335^{b} \pm 0.16$

USPS-Ultra sound priming seeds, CS-Control seeds, Mean (x±σ), $p \leq 0.05$

Results of the mesocotyl length (Table 1) demonstrated that this parameter of cereal seeds was affected by ultrasonication. In relation to the mesocotyl length it is noted that the largest value among sonicated seeds (5.525 cm ± 0.697) was recorded in rye, but not among control group (4.530 cm ± 1.110) which in turn have a very high variation in obtained values (3.0-5.9 cm). Variation within this crop is also the largest (from 4.1 to 6.5 cm). It has been recorded the shortest mesocotyl length among the ultrasound sonicated oat seeds (3.662 cm ± 0.411), as also the control (2.202 cm ± 0.312) whereas the smallest variation was evident (1.4-2.8 cm). Unlike the control, treated seeds are characterized by large variations in terms of 2.6-4.3 cm for the seed parameter. Seeds of *Triticale* developed long mesocotyl (5.180 cm ± 0.584) and among measurements is considered the large variation from 4.1cm to 6.5 cm. The control group is characterized by the longest mesocotyl (4.785 cm ± 0.862) and variations within wide range (3.1-5.8 cm). Shorter mesocotyl is ascertained among soft wheat seeds (4.822 cm ± 0.722), where priming of seeds with ultrasound has a small effect, but not one that could not be take into consideration. This crop among the control (4.335 cm ± 0.16) as well as ultrasound treated (3.8-4.5 cm) is emphasized by the smallest variation (4.0-5.9 cm).

From the analysis of variance for coleoptyle length presented in Table 2, according to the obtained p-values for F-ratio for the influence of ultrasound on the length of the coleoptile can be concluded that statistically significant difference

(95%) is the highest in *Triticale* (0.0016). Least significant mean (LSM) for coleoptile length showed positive and significant correlation with mesocotyl length (Table 3), whereas it was positively and highly significantly correlated (Table 4). Related to *Trtiticale* the statistically significant difference is determined for the length of mesocotyl (0.0076), allowing to conclude that ultrasound seed sonication has a significant effect on mesocotyl elongation (Table 5). In terms of least significant differences (LSD) among the investigated species highly significant difference was detected between *A.sativa* and *S.cereale* on the length of coleoptile (1.163) and mesocotyl (0.712) (Table 6 and 7). Although the coleoptile length depends on polygenic inheritance, seems that ultrasound priming of seeds has a very pronounced effect in all examined plants. Correlation coefficient (from ± 0.75 to ± 1) accordingly the used exponential model showed very high (0.821) and high R^2 (67,472) dependence among variables, both length of coleoptiles (as dependent variable) and mesocotyl (as independent) with low standard error (0.225).

Table 2. Analysis of variance for coleoptile length

Source	SS	Df	MS	F-Ratio*	P-Value
A: Species	15.023	3	5.007	36.52	0.0000
B: Ultrasound	10.823	1	10.823	78.92	0.0000
AB	2.860	3	0.953	6.95	0.0016
Residual	3.291	24	0.137		
Total (corrected)	31.997	31			

*All F-ratio

s are based on the residual mean square error.

Table 3. Least squares means for coleoptyle length (p≤0.05)

	Species	Count	LS Mean	LS Sigma	Homogenous Groups	Contrast	Sig.	Difference*	+/- Limits
1	*A.sativa*	8	2.159	0.131	X	1 - 2	*	-1.37	0.382
2	*S.cereale*	8	3.529	0.131	X	1 - 3	*	-1.755	0.382
4	*Triticale*	8	3.685	0.131	XX	1 - 4	*	-1.526	0.382
3	*Tr.aestivum*	8	3.914	0.131	X	2 - 3	*	-0.385	0.382
						2 - 4		-0.156	0.382
						3 - 4		0.229	0.382

*denotes a statistically significant difference

Table 4. Least significant differences for coleoptile length (p≤0.05)

Ultrasound	Count	LS Mean	LS Sigma	Homogeneous Groups	Contrast	Sig.	Difference	+/- Limits
2	16	2.74	0.092	X	1-2	*	1.163	0.270
1	16	3.903	0.092	X				

* denotes a statistically significant difference.

Table 5. Analysis of variance for mesocotyle length

Source	SS	Df	MS	F-Ratio*	P-Value
A: Species	26,0255	3	8,67516	31,09	0,0000
B: Ultrasound	4,05413	1	4,05413	14,53	0,0008
AB	4,21323	3	1,40441	5,03	0,0076
Residual	6,69602	24	0,279001		
Total (corrected)	40,9889	31			

*All F-ratios are based on the residual mean square error.

Table 6. Least squares means for mesocotyle length (p≤0.05)

Species	Count	LS Mean	LS Sigma	Homogenous Groups	Contrast	Sig.	Difference*	+/- Limits
1 *A.sativa*	1	8	2,9325	0,186749	1 - 2	*	-2,095	0,545
2 *S.cereale*	4	8	4,57875	0,186749	1 - 3	*	-2,292	0,545
4 *Triticale*	2	8	5,0275	0,186749	1 - 4	*	-1,646	0,545
3 *Tr.aestivum*	3	8	5,225	0,186749	2 - 3		-0,197	0,545
					2 - 4		0,449	0,545
					3 - 4	*	0.646	0,545

Table 7. Least significant differences for mesocotyle length (p≤0.05)

Ultrasound	Count	LS Mean	LS Sigma	Homogenous Groups	Contrast	Sig.	Difference*	+/- Limits
2	16	4.084	0.132	X	1 - 2	*	0.712	0.385
1	16	4.797	0.132	X				

*denotes a statistically significant difference

DISCUSSION

Even though scientiffic research reported coleoptile length is significantly affected by genotype and seed size, the interaction between these two factors are still under discussion (LIATUKAS. and RUZGA, 2011). Some of them (NEBREDA and PARODI, 1997; NIK *et al.*, 2011) suggested that coleoptiles length was highly correlated with seed size (that was the case here. Mesocotyl is controlled by genetic, developmental conditions and environmental signals as external influences and its length as result of elongation depend on the intensity and type of irritation/stimulation in case as it is the temperature (RADFORD and KEY, 1993; SAWERS *et al.*, 2002), light/darkness (CHOI *et al.*, 2003), enzyme activation during the seed germination. Ultrasound waves may result in physiological or biochemical changes in the seed that prime the germination process upon effect of the mentioned signals resulting in length of coleoptiles and mesocotyl. Also, ultrasound seed priming cause mechanical influence on seed cells by holes formation, allowing water penetration through them. This process of better hydration accelerated and induced releasing and activation of a higher amount of alpha-amylase speeding up the metabolic processes of starch degradation (DE CASTRO and CAPOTE, 2007).

From the aspect of global warming and evident climate change that led to the occurrence of drought and reduced yields, the application of seed priming with ultrasound could be useful. It is simple technique accomplished different elongation of cereal coleoptiles and mesocotyl. This achievement is particularly important because the faster growth of seminal roots, rooting and absorption of water and minerals provide greater opportunities for good seedling growth and further development of plants. The elongation of coleoptile provides shoot protection for a while before the emergence of the first leaves (SONEGO, 2000). Taking into account that this survey encompassed landraces, technique of ultrasonication could be recommended for traditional varieties which are shorter like soft wheat and rye align with previous research (REBETZKE *et al.*, 1999). In general, seed priming with ultrasound waves improve starting condition of plants, allowing more opportunities for normal growth and development and getting a good yield.

CONCLUSION

Length of coleoptile presents important feature as adapted mechanism especially nowadays when dealing with climate change. Within this study the priming of cereal seeds with ultrasound has been used to obtain the longer coleoptiles. Seedling screening was determined the longest coleoptile and mesocotyl in *Triticale*, although the coleoptile of the same length was recorded in rye. The shorter coleoptile was developed in soft wheat and the shortest one in oat. *Triticale* is appointed as crop with the highest statistically significant difference. Between *A.sativa* and *S.cereale* were detected highly least significant difference ($p \leq 0.05$) for the length of coleoptile (1.163) and mesocotyl (0.712). Using exponential model was established very high (0.821) and high R^2 (67,472) correlation among variables. Even the length of coleoptile depends on polygenic inheritance, seems ultrasound seed priming has a conspicuous effect in crops.

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UTICAJ ULTRAZVUKA NA DUŽINU KOLEOPTILA KOD KLIJANACA fam. *POACEAE* KAO ZNAČAJNOG PARAMETRA ZA PRIBRIDING I OPLEMENJIVANJE

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Studija istražuje efekat ultrazvuka na dužinu koleoptila i mezokotila kod klijanaca starih autohtonih sorata iz fam. *Poaceae*: ovas (*Avena sativa* L.) brodski, raž (*Secale cereale* L.) rakotinska, *Triticale* svetinikolsko i pšenica (*Triticum aestivum* L.) govrlevska. Tretmani su izvedeni sa frekvencijom od 30-40 kHz u termostatskom ultrazvučnom kupatilu na konstantnoj temperaturi od 25ºC u trajanju od 15 min. Ultrazvučni efekat se reflektuje na dužinu koleoptila i mezokotila, i pored toga što je njihov rast genetski predodredjen. Najduži koleoptil je izmeren kod *Triticale*-a (3.3-5.5 cm) i približne dužine kod raži (3.0-6.1 cm). Najduži mezokotil razvija raž (5.525 cm ± 0.697). Veća dužina koleoptila u semena tretiranom ultrazvukom ukazuje na brži razvoj klijanaca, bolji vodni režim i brži razvoj i pojavu prvih listova. Korelacioni kojeficient je vrlo visok (0.821) kao i R^2 (67,472%) što ukazuje na zavisnost varijabli dužina koleoptila (zavisna) i dužina mezokotila (nezavisna). Upotrebom ultrazvuka može se skratiti period klijanja, poboljšati vodni režim, a ranijim setvom dobiti zadovoljavajući prinosi i u uslovima globalnih klimatskih promena.

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