EFFECT OF DEUTERIUM DEPLETED WATER AND Pi WATER ABOUT *IN VITRO* GERMINATION OF MATURE CARYOPSES OF SOME SPECIES

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Abstract. In the present experiments we studied the capacity of three types of cereals – wheat (*Triticum aestivum* cv. Ariesan), rye (*Secale cereale* cv. Orizont) and their hybrid, triticale (*Triticosecale* Wittmack cv. Trilstar) - to be cultivated by specific methods based on vegetal biotechnologies, achieving "in vitro" cultures initiation, from mature caryopses, on culture mediums prepared with different types of water, respectively deuterium depleted water (with 25 ppm D) or Pi water, as the substitute of distilled water from the recipe elaborated by Murashige – Skoog (1962), by this observing the effects of these modifications in the composition of vitroculture medium, concerning the caryopsis germination. At the end of the experiment we found that, although in the first hours of germination, the deuterium depleted water, with 25 ppm (part per molecule) D – present in the culture medium – inhibits rootlets growing, at 5 days from the germination of three types of caryopses, on aseptic medium, substitution of distilled water, from the vitroculture medium, especially with deuterium depleted water, with 25 ppm D and in a smaller measure with Pi water, led to the significant stimulation, both of the germination process and plants growing resulted from germinated embryo, especially of its rootlets.

Keywords: cereals, micropropagation, deuterium depleted water, Pi water

INTRODUCTION

Water and cereals, raw materials in obtaining many food products, but not just, are two prime necessities of life, which can influence in direct way, by consuming them, or indirect way, the health and environment, in positive and negative way. Biotechnology offers many solutions for the problems which appeared in the last years, by using the resources inadequately, and by an irresponsible agriculture, using many chemical products, which had negative effects over the environment or health [22]. The agricultural and food biotechnological methods can improve the quality, safety, nutritive value and the variety of available foods for human consume and can increase the efficiency of food process and distribution.

For „in vitro" cultures water is an essential factor in the preparation of culture mediums, and, in their turn, the tissue and cell vegetal cultures represent a particularly valuable research tool in vegetal physiology, improvement and genetically studies, cellular and molecular biology studies, but in the mean time, it is a very modern methodology in obtaining different plant species with economical interest, at industrial scale [7]. Beside other specific elements seeds germination is a well studied physiological phenomenon, established unconditionally by water action.

Deuterium depleted water (D) produced by National Research-Development Institute for Cryogenic Technologies from Romania, residing at Râmnicu-Vâlcea, has similar composition as distilled water, but it contains lesser deuterium – a hydrogen isotope – as natural or distilled water [10].

The researches realized until the present time referring to the effect that can be exercised by deuterium depleted water regards the germination phenomena at three plant species, in natural life conditions, namely at wheat, corn and radish [2] or over "in vitro" for hyperhydricity annihilation [15], as well as in the exvitroplantlets acclimatization of chrysanthemum [14].

The second type of water used in our experiments, *Pi water* was produced basing on Bio Control System Technology, technologies elaborated and developed, 40 years ago, obtaining by purification and bioenergization of drinking water using Life Energy excellent removing harmful substances from water, Pi water getting physical and chemical properties [23].

The interest of Romanian botanist researchers for Pi water increased in the last years, their studies were materialized in brevet [4], as well as in other botanic and vegetal biotechnology researches [12, 13, 17].

Finding efficient and cheap solutions for the initiation of certain cereal „in vitro" cultures is an important element in the field of vegetal biotechnology, because, at present, there are modern cereal cultivation methods, by micropropagation. The most used cereal multiplication method is the one of meristem cultures of certain plantlets originated in germinated caryopsis in aseptically conditions [21] favorable because it excludes the eventual somaclonal variation, which can appear „in vitro" cultures via callus, as well as cultivation through microspores and immature pollen, from that embryos and finally mature plants, immature zygotic embryos vitroculture are formed, from which – under the influence of increasing regulators – callus is obtained, which, in its turn, on specific culture mediums will give a new plantlets; as part of these methods new variety of wheat or barley etc. can be obtained, by intervention at the level of genome [3, 9, 18].

MATERIALS AND METHODS

The three species used by us have great economic importance, they belong to *Monocotyledonopsida* class, *Graminalis* order, *Gramineae* family, *Triticum (T. aestivum* cv. Ariesan*)*, *Secale* (*S. cereale* cv. Orizont*)* types and a new type, created by man by hybridizing the *Triticum* and *Secale*, where triticale hybrid

(Triticosecale Wilttmack cv. Trilstar) belongs to (being an *amphydiploid* between wheat and rye, from genetic view point, with useful features in agricultural production) [1].

We used the basic culture medium (MB) Murashige-Skoog (MS) [8], in ½ concentrations, without growth regulators, prepared with three types of water, namely:

V_0 – MB - MS prepared with *distilled water* (DW) – control;

V_1 – MB - MS prepared with *deuterium depleted water*, with *25 ppm D* (DDW);

V_2 – MB - MS prepared with *Pi water* (PiW).

After preparation and adjustment of pH at value 5.5, the culture medium was divided, each 5 ml medium, in culture recipients of colorless, thermal resistant bottle, with a 7 cm height and 2 cm diameter. The culture medium and water sterilization, needed in caryopsis aseptization was obtained by autoclaving at 121˚C (1 atm.), for 20 minutes.

For seed aseptization, the seeds were submersion for 30 minutes, in sodium hypochlorite, with an active chlorine content of 16.7 %, procured from trade, to which were added a few drops of Tween 20. The whole disinfection action took place in hood under laminar, horizontal flow of sterile air, in action.

After inoculation, the culture vessels were closed with colorless polyethylene thin sheet and were transferred on shelves in the growth room, being illuminated with white fluorescent tubes, with an 1700 lx intensity, a16 h light/24 h photoperiod: the cultures were kept on varied temperature between 23 ºC ± 2ºC, in light period and 20ºC ± 2ºC in dark period.

RESULTS

At 24 hours from putting the caryopses to germinated, in aseptic conditions, the highest germination faculty was registered at the wheat caryopses germinated on mediums prepared with DDW and PiW, as well as at the triticale, with all types of culture mediums (Table. 1).

Table 1. The caryopsis germination faculty at 24 hours from their putting to germinate on aseptic, on the following culture mediums: V_0 – culture medium MS, prepared with distilled water (DW), V_1 – culture medium MS, prepared with deuterium depleted water (DDW) or V_2 – culture medium MS, prepared with Pi Water (PiW).

Species / Variants	*Wheat* %	*Rye* %	*Triticale* %
V_0 (DW)	89	85	98
V_1 (PiW)	97	83	98
V_2 (DDW)	98	70	93

At 5 days from putting the wheat *(Triticum aestivum* cv. Ariesan*) caryopses to germinated on aseptic mediums, the highest average length of embryonic roots, 2,4 cm, was registered at vitroplantlets belonging to the cultivated lots on mediums prepared with DDW (V_1) and with Pi water (V_2) (Table 2), but insignificant from statistical point of view (Table. 2).

Table 2. The statistical processing of biometrical data to wheat (*Triticum aestivum* cv. Ariesan*)* vitroplantlets level, at 5 days from putting the caryopses to germinated on the following culture mediums: V_0 – culture medium MS, prepared with distilled water (DW), V_1 – culture medium MS, prepared with deuterium depleted water (DDW) or V_2 – culture medium MS, prepared with Pi Water (PiW). The percentage values (%) of variants V_1 and V_2 is obtained in propotion to the biometrical data at control lot plantlets, germinated on medium prepared with *distilled water* (V_0 – DW), values considered as being 100%.

Type V_0 - *control*

Statistical calculations	Rootlet length	%	Coleoptile length	%	Leaflet length	%	Plantlet size	%
X ± Sx	2.11±0,10	100	1.46±0,14	100	1.50±0,17	100	5.07±0.30	100
S	0,54		0.77		0.93		1.66	
S%	26%		53%		62%		33%	

Type V_1 - DDW

X ± Sx	2.46±0,13	118	1.58±0.14	110	1.18±0.14	80	5.22±0.33	105
S	0.74		0.81		0.80		1.86	
S%	30%		51%		67%		36%	
±d	0.35		0.12		-0.32		0.15	
P	ns		ns		ns		ns	

Type V_2 – PiW

X ± Sx	2.45±0.09	117	1.77±0.13	120	1.38±0.13	90	5.60±0.24	110
S	0.46		10.68		0.65		1.21	
S%	19%		39%		47%		22%	
±d	0.35		0.31		-0.12		0.53	
p	ns		*		ns		ns	

Note: $\overline{X} \pm S_{\overline{x}}$ (average (cm) ± standard average deviation), s. (standard deviation), S% (variability value), ±d (difference compared to the control – in cm), p (significance level of difference compared to the control), ns (no significant difference), * - significant difference, ** - distinct significant difference, *** - very significant difference.

Positive but insignificant differences compared to the control were also identified at the two tested lots and in the case of length average of coleoptiles, of leaflets number and vitroplantlets size (Table 2).

Concerning the fresh weight it was identified one single higher value than the control lot, 0.6544g, with 112% (Table 3), in the case of vitroplantlet lot resulted from the mature wheat caryopses embryos and grown for 5 days on mediums prepared with deuterium depleted water DDW (V_1). If it was registered a plus at the V_1 variant concerning the fresh weight, after drying for 3 days on the sterilizer, at 115ºC temperature, the vitroplantlets dry weight belonging to this variant presented a minus of 10% compared to the measured lot resulted from mediums prepared with DW (V_0) (Table 3).

Table 3. Fresh weights, measured at 5 days from the culture initiation of wheat (*Triticum aestivum* cv. Ariesan), rye (*Secale cereale* cv. Orizont*)* or triticale *(Triticosecale* Wittmack cv. Trilstar*)* caryopsis, on aseptic mediums prepared with *distilled water* (DW), *deuterium depleted water, with 25 ppm D* (DDW) or *Pi water* (PiW) and their dry weights, registered after putting the fresh mass to the sterilizer, at 115 ºC temperature, for 3 days. The percentage values (%) of variants V_1 and V_2 is obtained in propotion to the biometrical data at control lot plantlets, germinated on medium prepared with *distilled water* (V_0 – DW), values considered as being 100%.

Biometrics / Variants	Fresh weight						Dry weights					
	Wheat		*Rye*		*Triticale*		*Wheat*		*Rye*		*Triticale*	
	g	%	g	%	g	%	g	%	g	%	g	%
V_0 (DW)	0.5837	100	0.882	100	0.4868	100	0.0742	100	0.0743	100	0.0501	100
V_1 (DDW)	0.6544	112	0.9524	108	0.7842	161	0.0667	90	0.0771	104	0.0871	174
V_2 (PiW)	0.5725	98	0.7453	85	0.558	115	0.0552	74	0.0702	94	0.057	114

At 5 days from putting the rye *(Secale cereale* cv. Orizont*)* caryopsis to germinated, concerning the rootlets length, from mature rye caryopsis put on culture mediums prepared with DW (V_0), DDW (V_1), respectively PiW (V_2), rootlets were formed which registered an insignificant minus in the case of germinated vitroplantlets on culture mediums in which distilled water (control – V_0) was replaced with deuterium depleted water (DDW – V_1) and also a minus was registered, 0.57 cm, at the V_2 lot in which the distilled water was replaced with PiW, a significant value from statistical point of view (Table. 4). The caulogenesis, expressed by the coleoptile length, generated pluses, both positive differences but insignificant statistically (Table 4), nevertheless, in the case of V_1 (DDW), this plus was expressed in percentage by relatively high values, 131% (Table 4).

The leaflet length average was insignificantly low compared to the control (V_0), at both lots treated with different types of water (DDW and PiW) (Table 4).

Directly proportional to mediums value of growth indications it was situated the plantlets size, this recording positive differences (in the case of V_1 variant), or negative differences (in the case of V_2 variant), but both insignificant compared to the control from the perspective of statistical process.

The pluses registered at the growth indications concerning the coleoptile length and the vitroplantlet whole size, recorded at the plantlet lot grown on medium prepared with DDW (V_1) was reconfirmed by a high fresh weight, by 0.9524 (Table 3), which represents a 108% growth, compared to the control (V_0).

Table 4. The statistical processing of biometrical data to rye (*Secale cereale* cv. Orizont*)* vitroplantlets level, at 5 days from putting the caryopses to germinated on the following culture mediums: V_0 – culture medium MS, prepared with distilled water (DW), V_1 – culture medium MS, prepared with deuterium depleted water (DDW) or V_2 – culture medium MS, prepared with Pi Water (PiW). The percentage values (%) of variants V_1 and V_2 is obtained in propotion to the biometrical data at control lot plantlets, germinated on medium prepared with *distilled water* (V_0 – DW), values considered as being 100%.

Type V_0 – *control*

Statistical calculations	Rootlet length	%	Coleoptile length	%	Leaflet length	%	Plantlet size	%
X ± Sx	3.84±0.19		2.26±0.162		2.24±0,15		8.52±0.44	
S	0,97	100	0.82	100	0.76	100	2.22	100
S%	25%		36%		31%		26%	
Type V_1 – DDW								
X ± Sx	3.77±0.16		2.97±0.14		2.25±0.18		8.99±0.40	
S	0.83		0.73		0,92		2.03	
S%	22%	98	25%	131	41%	86	23%	106
±d	-0.07		0.71		-0.17		0.47	
P	ns		*		ns		ns	
Type V_2 – PiW								
X ± Sx	3.27±0.15		2.33±0.17		2.27±0.18		7.88±0.41	
S	0.74		0.81		0.86		1.97	
S%	23%	87	35%	105	38%	87	25%	87
±d	-0.57		0.07		-0.14		-0.64	
P	*		ns		ns		ns	

Note: $\overline{X}$ ± S $\overline{x}$ (average (cm) ± standard average deviation), s. (standard deviation), S% (variability value), ±d (difference compared to the control – in cm), p (significance level of difference compared to the control), ns (no significant difference), * - significant difference, ** - distinct significant difference, *** - very significant difference.

At 5 days from putting to germinated the triticale (*Triticosecale* Wittmack cv. Trilstar*)* caryopses, the rootlet length registered positive differences, of 0.68 cm and 0.19 cm, in the first case they being significant from statistical view point, and in the case of cultivated lot on PiW (V_2) they were distinct insignificant (Table 5), expressed in percentage of 142%, respectively 111% .

The longest coleoptiles, 1,74 cm were biometrized at vitroplantlets grown for 5 days on mediums prepared with DDW (V_1), followed by those grown on mediums with PiW (V_2), both recording clear positive differences compared to the control (V_0) (Table 5), these pluses expressed in percentage were 158%, respectively only 108%.

Leaflets were also identified, the longest, 0.99 cm, measured at the V_1 variant level (DDW), insignificant dimensions from statistical point of view compared to those at the control lot, but the growth, expressed in percentage, was increased, to 57% (Table 5), the same

Table 5. The statistical processing of biometrical data at triticale (*Triticosecale* Wittmack cv. Trilstar*)* vitroplantlets level at 5 days from putting the caryopses to germinated on the following culture mediums: V_0 – culture medium MS, prepared with distilled water (DW), V_1 – culture medium MS, prepared with deuterium depleted water (DDW) or V_2 – culture medium MS, prepared with Pi Water (PiW). The percentage values (%) of variants V_1 and V_2 is obtained in propotion to the biometrical data at control lot plantlets, germinated on medium prepared with *distilled water* (V_0 – DW), values considered as being 100%.

Type V_0 - *control*

Statistical calculations	Rootlet length	%	Coleoptile length	%	Leaflet length	%	Plantlet size	%
X ± Sx	1.60±0,12		1.09±0,10		0.63±0.06		3.32±0,25	
S	0.45	100	0.39	100	0.25	100	0.98	100
S%	28%		36%		39%		30%	
Type V_1 – DDW								
X ± Sx	2.28±0,19		1.74±0.15		0.99±0.12		5.01±0.40	
S	0.78		0.64		0.48		0.65	
S%	34%	142	37%	158	49%	160	33%	150
±d	0.68		0.64		0.36		1.69	
P	***		**		ns		***	
Type V_2 – PiW								
X ± Sx	1.79±0.09		1.19±0.08		0.78±0.07		3.75±0.20	
S	0.41		0.35		0.32		0.95	
S%	23%	111	30%	108	41%	120	25%	118
±d	0.19		0.09		0.15		0.43	
P	**		**		ns		**	

Note: $\overline{X} \pm S_{\overline{x}}$ (average (cm) ± standard average deviation), s. (standard deviation), S% (variability value), ±d (difference compared to the control – in cm), p (significance level of difference compared to the control), ns (no significant difference), * - significant difference, ** - distinct significant difference, *** - very significant difference.

situation was in the case of plantlets grown on PiW, but superiority of this indication was only 24% (Table 5).

The high values of embryonic rootlets length, of coleoptiles and leaflets led to a high size of the whole plantlet, to both vitroplantlet lots which were grown on mediums where the distilled water was replaced with other types of water, reaching values of 5.01 cm, in the case of mediums prepared with DDW (V_1), respectively of 3.75 cm, at those come from mediums with Pi water (V_2) content, recording positive differences, significant or distinct significant from statistical point of view, compared to the control (V_0) (Table 5).

The fresh weight, reaching of 0.7842 in the case of cultivated lot on DDW (V_1), strengthens the superior growth of plantlets on these culture mediums, compared to the control (V_0) (Table 3). Directly proportioned to the fresh weight dry weight growths were identified, these being 174%, at V_1 variant (DDW) and 114%, at V_2 variant (PiW).

DISCUSSIONS

Unlike wheat *(Triticum aestivum* cv. Ariesan*)* and triticale (*Triticosecale* Wittmack cv. Trilstar), at rye *(Secale cereale* cv. Orizont*),* the two types of water – especially deuterium depleted water (with 25 ppm D) – had inhibitory effect over the beginning of germination process, compared with distilled water. The germination of cereals depends by the genotype and the age of caryopses. Optimum age of the embryo for better recovery of complete plantlets coincided with onset of the drying of hybrid caryopses in the field [6]. ”In vitro” condition, was reported 55.55% the best embryo germination, and plantlet recovery 50%, recorded in the hybrid 'HD 2380' wheat x 'JNIT 173' triticale [6]. Our germination percentage was better. Some basic information regarding the influence of stage of kernel maturation and temperature during kernel maturation on sprouting tolerance of triticale (*Triticosecale* Wittmack cv. Trilstar) and wheat (*Triticum aestivum* cv. Ariesan) was studied by compared ”in vitro” germination tests (at 17°C or 25°C), and by rain simulation. From these results it was concluded that the 25°C ”in vitro” germination test provided a suitable alternative for the rain-simulator facility that may not always be available [16]. The germination test also produced tolerance ratings that consistently differentiated among genotypes over a range of stages of kernel development as well as different temperatures during kernel maturation.

In our experiments, in the case of grown indications of organs generated by embryos, namely the length of embryonic rootlets, of coleoptiles and fresh weight, at wheat and triticale the two types of lot whose caryopsis germinated on mediums where the distilled water was replaced with one of the two types of water, the values were superior than those biometrized at the level of control lot. The replacement of distilled water, from vitroplantlet mediums, especially with deuterium depleted water, with 25 ppm D, and in a smaller extent with Pi water, is a relatively cheap and available procedure for anybody, for protocol improvement of cereal micropropagation, leading to the significant stimulation, both of germination process and to the plants growth resulted from germinated embryos, especially of rootlets (even if, in the first hours after germination is recorded an inhibition of vitroplantlet growth).

The triticale caryopses are adapted very well to „in vitro” culture initiation, like the wheat and rye caryopses, and the „in vitro” growth at the triticale was higher than the examples belonged to its sire parents. At triticale, breeding effort has increased yield, reduced shriveling and improved test weight but at the expense of protein content, which is now comparable to wheat and rye [11]. Plant height and lodging are also now comparable to wheat and rye. Progress in reducing

preharvest sprouting by genetic selection is proving difficult and slow. On triticale there is more study for "in vitro" amelioration of hybrids, because most negative effect on seed germination is soil salinity [19]. So, the effects of seed priming treatments with 0.5% KH_2PO_4 (w/v) solution and water were determined on germination and seedling characters of hexaploid triticale (*Triticosecale* Wittmack cv. Presto) in different osmotic potential (-0.45, - 0.77, -1.03 and -1.44 MPa and control) of NaCl and PEG 6000 solutions [20]. Despite the negative effects of two stress conditions, the two priming treatments were effective in improving germination percentage and seedling growth. But seed primed treatment was effective at the lowest osmotic potentials; therefore, seedling growth survived at the highest concentrations. The effect of hydropriming was very pronounced particularly in improving germination and seedling growth in low stress [20]. Hydropriming method has also been used successfully in wheat [5].

REFERENCES

[1] Bălteanu, G., (1993): Phytotechnique, Vol.II. Ceres Press, Bucharest, 235 p.

[2] Cachiță-Cosma, D., Petruş, C.M., Vancea, A., Ardelean, A., Moraru, V., Ştefănescu, I., (2002): The deuterium depleted water effect about wheat, corn and radishes germination. „Water, Environment and Helth", EASA Conference, Arad, pp. 83-86.

[3] Greer, M.S., Kovalchuk, I., Eudes, F., (2009): Ammonium nitrate improves direct somatic embryogenesis and biolistic transformation of *Triticum aestivum.* New Biotechnology Volume 26 (1), Special issue on Biocatalysis and Agricultural Biotechnology: 44-52.

[4] Godeanu Marioara, Stanca Doina, Stanescu Ioana (1997): Forma de *Spirulina platensis* (Nordst) Geitl si mediu de cultura pentru obtinerea de biomasa. Patent RO 117387.

[5] Harris D, Raghuwanshi BS, Gangwar JS, Singh SC, Joshi KD, Rashid A, Hollington PA (2001): Participatory evaluation by farmers of on-farm seed priming in wheat in India, Nepal, and Pakistan. Experimental Agriculture, 37: 403-415.

[6] Kapila, R.K., Sethi, G.S., (1993): Genotype and age effect on in vitro embryo rescue of bread wheat x hexaploid triticale hybrids. Plant Cell, Tissue and Organ Culture, 35 (3): 287-291.

[7] Moris, P.C, James, J.B., (2000): Cereal biotechnology. Wood head Publishing Limited, Cambridge, 251 p.

[8] Murashige, T., Skoog, F., (1962): A revised medium for rapid growth bioassays with tobacco tissue cultures. Physiologia Plantarum, 15: 473-497.

[9] Nehra, N.S., Chibbar, R.N., Leung, N., Caswell, K., Mallard, C., Steinhauer, L., Baga, M., Kartha, K.K., (1994): Self-fertile transgenic wheat plants regenerated from isolated scutellar tissues following microprojectile bombardment with two distinct gene constructs. Plant Journal, 5(2): 285-297.

[10] Nuțiu, R., Ardelean, D., (2002): Water Reverse and Chemistry. „Vasile Goldiş" University Press, Arad, 74 p.

[11] Oettler, G., (2005): The fortune of a botanical curiosity – Triticale: past, present and future. The Journal of Agricultural Science, 143(5): 329-346

[12] Petruş, C.M., Petruş-Vancea, A., (2004): Rooting of *Tradescantia* L. cuttings watering them with Pi water. Analele Universității din Oradea – Fascicula Biologie, Tom. XI: 187-189.

[13] Petruş, C.M., Petruş-Vancea, A., Zahan, A., (2004): Effect of Pi water on wheat, corn and radishes germination. Analele Universității din Oradea – Fascicula Biologie, Tom. XI: 183-186.

[14] Petruş-Vancea, A., Cachiță-Cosma, D., Blidar, C.F., Ştefănescu, I., (2003): The effect of dedeuterised water in acclimatization of *Chrysanthemum* vitroplantlets to septic medium. Papers of the 5th International Symposium Young People and Multidisciplinary Research, Sudura Press, Timişoara, pp. 335-340.

[15] Petruş-Vancea, A., Radoveț-Salinschi, D., Cachiță-Cosma, D., (2008): Hiperhydricity annihilation out of vitrocultures with deuterium depleted water and pi water, using a double layer system. Proceeding of the International Symposium "New research in biotechnology", Series F, Biotechnology, Special volume: 20-30.

[16] Plett, S., Larter, E.N., (1986): Influence of Maturation Temperature and Stage of Kernel Development on Sprouting Tolerance of Wheat and Triticale. Crop Science, 26: 804-807.

[17] Radoveț-Salinschi, D., (2004): The Effect of „Pi" Water Over Hyperhidric *Coleus blumei* Benth. vitroplants. Papers of the 6th International Symposium Young People and Multidisciplinary Research. Sudura Press, Timişoara, pp. 499 – 506.

[18] Weeks, J.T., Andersen, O.D., Blechl, A.E., (1993): Rapid production of multiple independent lines of fertile transgenic wheat (*Triticum aestivum* L.). Plant Physiology, 102: 1077-1084.

[19] Yahmur M, Kaydan D, Okut N (2007). Alleviation of salinity stress during seed germination in wheat (*Triticum aestivum*) by potassium applications. Indian Journal of Agricultural Sciences, 77(6): 379-882.

[20] Yahmur, M., Kaydan, D., (2008): Alleviation of osmotic stress of water and salt in germination and seedling growth of triticale with seed priming treatments. African Journal of Biotechnology, 7 (13): 2156-2162.

[21] Zhang, S., Cho, M.J., Koprek, T., Yun, R., Bregitzer, P., Lemaux, P.G., (1999): Genetic transformation of commercial cultivars of oat (*Avena sativa* L.) and barley (*Hordeum vulgare* L.) using *in vitro* shoot meristematic cultures derived from germinating seedlings. Plant Cell Reports, 8: 959-966.

[22] Zinnen, T., Voichick, J., (1994): Biotechnology and Food. pp. 234-240. In: Madison, W.I. (ed.): Cooperative Extension Publications. University of Wisconsin – Extension, North Central regional Extension Publication 569.

[23] http://www.pi-water.org/, accessed in 10 March 2010.

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