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Full Length Article

Assessment of Somaclonal Variation in Regenerated Plants from Immature Embryos Culture of Durum Wheat

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ABSTRACT

Somaclones, regenerated from three durum wheat cultivars Karim, Sebou and Isly, were evaluated for various agronomic and morphological characters. Calli were initiated from immature embryos on modified Murashige and Skoog medium containing 2 mg L^{-1} of 2, 4-D and regenerated on media supplemented with BAP (10 µM) and NAA (5 µM). Calli with shoot were transferred to a hormone-free half-strength MS medium. The seedlings with well-developed roots were transplanted into pots containing a substrate and were acclimatized. After acclimation, plantlets were transplanted to field conditions. Most of the regenerated plants were phenotypically normal. However, some plantlets were albino, with deformed leaf or with abnormalities in spike morphology. Variation was recorded in the R1 generation. Comparison were made between the somaclones and their parents (non-tissue-cultured plants) for plant height, days to heading, 100-grain weight, grain number per spike and number of fertile tillers per plant. Comparison for agronomic and morphological characters between the parents and somaclones and among the somaclones revealed significant differences. However, the parents showed superiority over somaclones for important agronomic traits.

Key Words: Tissue culture; Somaclonal variation; *Triticum durum*; Somatic embryogenesis; Plant regeneration; Albinism
Abbreviations: 2,4-D: 2,4-Dichlorophenoxyacetic acid; NAA: Naphthalene acetic acid; BAP: 6-Benzylaminopurine.

INTRODUCTION

The term somaclonal variation was first used by Larkin and Scowcroft (1981) to describe induced changes in plants regenerated from *in vitro* cultured somatic tissues. These variations are considered as a new source for enlarging and enriching the gene pool of improved varieties (Evans & Sharp, 1988; Bairu *et al.*, 2011). Currently, the term somaclonal variation is generally used to describe all the variations from tissue culture. However, other alternative terms such as 'protoclonal' or 'gametoclonal', describing variation from protoplast and anther culture, respectively, are also used (Karp, 1994). Somaclonal variation is an important phenomenon that can be observed at varying levels in plant tissue culture in both dicot (Brar & Jain, 1998) and monocot families (Cheng *et al.*, 1992; Chang *et al.*, 2003). The frequency of occurrence of somaclonal variation in regenerated plants is strongly affected by genotype (Breiman *et al*., 1987), medium and duration of culture (Cassells & Curry, 2001; Jain, 2001) and the source of explant (Ahloowalia & Sherington, 1985). Somaclonal variation has been observed in many cereal species such as wheat (Larkin *et al*., 1984; Ahloowalia & Sherington, 1985), maize (Edallo *et al.*, 1981), rice (Kabir *et al*., 2008) and barley (Li *et al*., 2007). Somaclonal variation has been reported in wheat for various traits such plant height, spike length and leaf size (Ahloowalia, 1982; Carver & Johnson, 1989; Cheng *et al.*, 1992); number of grains per spike and grain weight (Ahloowalia & Sherington, 1985; Arun *et al*., 2003); early maturity, high yield and superior quality (Liang *et al*., 1996); frost tolerance (Dorffling & Melz, 1997) and disease resistance (Arun *et al*., 2003; 2007).

Frequently encountered types of somaclonal variation result from changes in chromosome number (aneuploidy, polyploidy or mixoploidy), abnormal structural changes of chromosomes (deletion, addition, transposition or inversion), single-gene mutations (Larkin & Scowcroft, 1981; Lörz *et al*., 1988) and DNA methylation (Kaeppler *et al*., 2000). Many authors have reported that the cytoplasmic genome could be responsible for certain variations. Indeed, mutations in mitochondrial DNA and chloroplast cause regeneration of albino plants and cytoplasmic male sterility (Larkin & Scowcroft, 1983). These variations are apparently the result of genetic changes that occur during the time the calli are in culture, however, somaclonal variation is also attributed to pre-existing genetic variation in somatic cells (Evans *et al*., 1984).

The exploitation of heritable somaclonal variants has been used in various plant improvement strategies. Somaclonal variation can be exploited by looking in the regenerated plants that might be carrying interesting

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heritable traits (Evans, 1989). However, methods more *in vitro* selection directed can exploit this change by exerting a selective pressure targeted to improve the tolerance of biotic or non-biotic stress (Lestari, 2006).

In this study, we attempted to assess the degree of variation obtained *in vitro* regenerated plants of three durum wheat genotypes known for their good *in vitro* culture with focus on phenotypic traits observed *in vitro*, and agronomic traits studied in the field to identify the possible genotypes that can later be integrated into a breeding program.

MATERIALS AND METHODS

Plant material: Three durum wheat (*Triticum durum* Desf.) cultivars, Karim, Sebou and Isly were used as experimental material in present work. The seeds were provided by INRA (National Institute for Agricultural Research, Morocco). The explant source consisted of immature embryos (about 0.5-1.5 mm long), collected from seeds in the milky phase, approximately 14-16 days after anthesis. The caryopses were surface sterilized for 1 min in 90% ethanol and rinsed three times in sterile distilled water. Caryopses were disinfected again with 20% commercial bleach for 20 min followed by three rinses with sterile water.

Callus induction: The immature embryos were excised aseptically from caryopses, and placed with the embryo axis in a contact with a solid agar medium for callogenesis containing the mineral salts of Murashige and Skoog (1962) 30 g L^{-1} sucrose and 2 mg L^{-1} 2,4-D. The pH of this medium was adjusted to 5.8 with 0.1 N NaOH and solidified with 7 g L^{-1} agar before autoclaving during 20 min at 120°C. Ten explants per petri dish were cultivated and cultures were kept in dark at 25±1°C.

Plant regeneration: All calli produced by the embryos were transferred to jars containing a regeneration medium after 6 weeks of culture. The regeneration medium (MR1) consisted of MS basic medium, supplemented with 0.2 mg $L^{-1}$of 2,4-D, 5 µM naphthalene acetic acid (NAA) and 10 µM benzyl aminopurine (BAP). Regenerated shoots were rooted on half strength MS medium in the absence of growth regulators (MR2). The cultures were incubated in a growth room, with light conditions fixed by fluorescent lights at a photoperiod of 16 h light/8 h dark at 25±1°C.

Acclimation of regenerated plants: Plantlets with at least five well-developed roots were subjected to acclimation, transplanted to potting soil under high humidity by covering the plant with plastic envelopes. Pots were placed in growth chamber at 25±1°C under 16 h photoperiod. After acclimation, plantlets were transplanted to field conditions.

Determination of somaclonal variation: Seedlings grown in MR2 medium were regularly checked for the presence of variant phenotypes. Emphasis was placed mainly on morphological characteristics such as albinism and shoot morphology such as leaf abnormality. After transfer to soil, plants grown in pots were regularly checked for the presence of variant phenotypes. The study mainly focused on morphological characteristics such as spike morphology and leaf characteristics.

Studies of R1 somaclones were conducted in a randomized complete block design with 16 treatments consisting of three parents and thirteen populations with four replications of 60 plants each. The plants were evaluated for plant height, days to heading, 100-grain weight, grain number per spike and number of fertile tillers per plant.

Statistical analysis: Data were analyzed using the ANOVA procedure of R statistical environment (R Development Core Team, 2012). Means were compared by Duncan's multiple range test at P = 0.05.

RESULTS

Regenerated plants before their transfer to soil revealed seedlings with morphological changes, including shoots with deformed leaf, albino seedlings and rarely chimerical plantlets with leaves in albino and green bands (Fig. 1). The regeneration of albino plantlets was observed in both genotypes Karim and Isly (Table I). No albino plants were regenerated from a Sebou callus. On the other hand, chimerical seedlings with foliar malformations were observed in all three genotypes. After transfer to ground, most F0 seedlings, monitored at maturity, showed a normal phenotype. However, phenotypic variations were recorded and involved mainly spike morphology and fertility (Fig. 2a, b & c). Nevertheless, R1 plants, resulting from R0 that present abnormality in spike morphology, did not show any spike defect. The study of the variations observed in R1 somaclones (Fig. 2d) showed significant differences in most studied characters.

Plant height: Significant differences were recorded amongst the somaclones and their parents in the three studied varieties (Tables I-III). In Karim and Isly, the parents showed the highest values for plant height compared to their somaclones. The height varied in Karim from 91.4 cm in the parent to 84.6 cm in the Kar-SOM1c, and in Isly from 99.2 in the parent to 89 in the Isly-SOM1a. In Sebou, the recorded height was lower in the parent (91.7 cm) than in the Seb-SOM1d (95.7 cm).

Days to heading: The somaclones and parents flowered at different intervals (Tables I-III) and significant differences between the somaclones and their parents were observed. The parent Karim differed significantly from all somaclones (Kar-SOM1) with 109.3 days to flowering. In Sebou it is the Seb-SOM1d that differed from the parent and other somaclones (Seb-SOM1) with the lowest number of days to flower (94.6). In Isly, the lowest number of days to flowering (77.5) was observed in Isly-SOM1c; and the highest (83.7) was recorded for Isly-SOM1d.

100-grain weight: Data **(**Tables I-III) showed significant differences in 100-grain weight for somaclones for all varieties studied, and parents had the highest weight.

Table I: Regeneration of green, albino and abnormal-leaf-morphology plants from immature embryos derived calli of three durum wheat genotypes

Genotype	Regeneration (%)	Number of regenerated shoots				
		Total	Normal	Albino	Abnormal leaves	Chimeric
Karim	89.99 ± 1,92	445	399	18	16	12
Sebou	88.88 ± 1,11	319	298	0	13	8
Isly	73.33 ± 3,85	309	276	10	19	4

Table II: Variation in agronomic and morphological characters of variants (R1) derived from Karim

Variant lines	Plant characters				
	Plant height (cm)	Days to heading	100-grain weight (g)	GNPS*	NFTP*
Parent	91.4 a	109.3 a	3.96 a	48.3 a	4.6 a
Kar-SOM1a	85.1 b	103.5 b	3.92 a	47.5 a	3.9 ab
Kar-SOM1b	87.5 ab	97.6 c	3.89 ab	44.8 ab	4 ab
Kar-SOM1c	84.6 b	94.1 c	3.84 abc	42.7 b	4 ab
Kar-SOM1d	85.1 b	98 c	3.67 c	46.5 ab	3.9 b
Kar-SOM1e	89.9 a	98.2 c	3.72 bc	44.5 ab	4.3 ab

Means within columns followed by the same letter are not different (Duncan's multiple range test, P<O.05); *GNPS: Grain number per spike; *NFTP: Number of fertile tillers per plant

Table III: Variation in agronomic and morphological characters of variants (R1) derived from Sebou

Variant lines	Plant characteristics				
	Plant height (cm)	Days to heading	100-grain weight (g)	GNPS*	NFTP*
Parent	91.7 bc	105.5 a	3.90 a	51.8 a	3.5 b
Seb-SOM1a	90.2 bc	102.3 a	3.89 ab	49.8 abc	3.8 ab
Seb-SOM1b	89.7 c	103.7 a	3.84 ab	50.9 ab	4.1 a
Seb-SOM1c	93.5 ab	103.7 a	3.84 ab	47.9 c	4.1 ab
Seb-SOM1d	95.7 a	94.6 b	3.79 b	48.7 bc	3.7 ab

Means within columns followed by the same letter are not different (Duncan's multiple range test, P<O.05); *GNPS: Grain number per spike; *NPTP: Number of fertile tillers per plant

Table IV: Variation in agronomic and morphological characters of variants (R1) derived from Isly

Variant lines	Plant characteristics				
	Plant height (cm)	Days to heading	100-grain weight (g)	GNPS*	NFTP*
Parent	99.2 a	80.8 ab	3.66 a	44,9 a	4.5 ab
Isly-SOM1a	89 b	79.9 bc	3.49 b	40.6 c	4.2 b
Isly-SOM1b	92.8 b	78.2 bc	3.74 a	42.7 abc	4.4 ab
Isly-SOM1c	89.4 b	77.5 c	3.38 b	41.7 bc	4.3 ab
Isly-SOM1d	92.5 b	83.7 a	3.68 a	44.3 ab	4.7 a

Means within columns followed by the same letter are not different (Duncan's multiple range test, P<O.05); *GNPS: Grain number per spike; *NPTP: Number of fertile tillers per plant

For the Sebou variety, only somaclone Seb-SOM1d differed significantly from the parent with 3.79 g weight of 100 grains. For genotype Isly, it is the somaclones Isly-SOM1a and Isly-SOM1c that differed from the parent (3.66 g) with 3.49 g and 3.38 g 100-grain weight, respectively. Regarding Karim, the somaclones Kar-SOM1d and Kar-SOM1e had the lowest weight of grains and were significantly different from the parent Karim with 100-grain weight of 3.67 g and 3.72 g respectively.

Grain number per spike: The number of grains per spike was highest for parents in the three varieties. This number was 48.3 in Karim, 51.8 in Sebou, and 44.9 in Isly (Tables I-III). One somaclone differed significantly from parents of Karim (namely Kar-SOM1c) and two somaclones in the case of the variety Sebou (namely Seb-SOM1a & Seb-SOM1c). In Isly, the somaclones Isly-SOM1a and Isly-SOM1c differed significantly from the parent with 40.6 and 41.7 grains per spike, respectively.

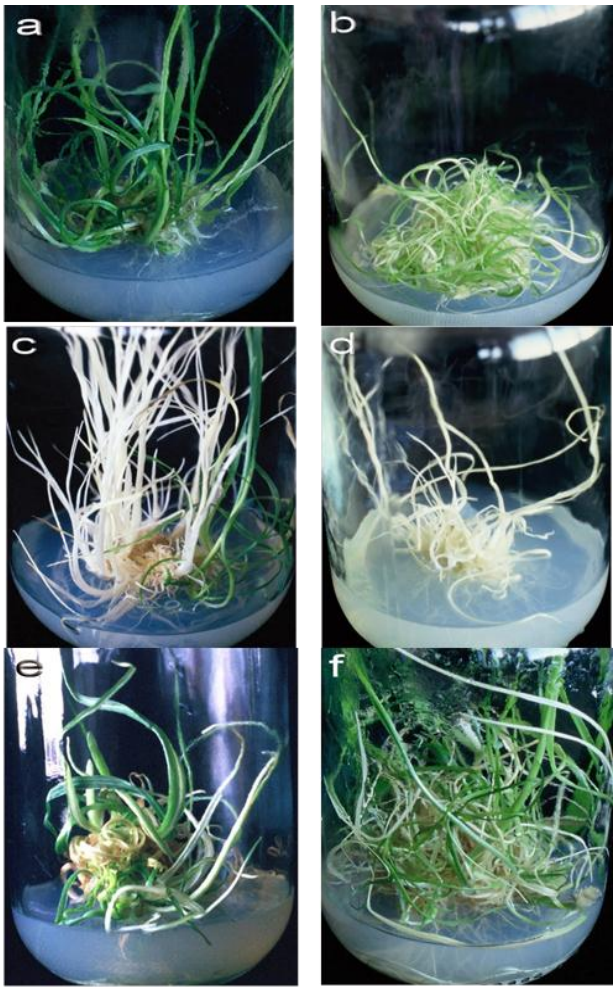
Number of fertile tillers per plant: Results (Tables I-III) showed significant differences in the number of fertile tillers per spike among parents and their somaclones. The best results for this character were observed in somaclones Seb-SOM1b from Sebou and Isly-SOM1d from Isly, with 4.1 and 4.7 fertile tillers per plant, respectively. As for Karim, the parent observed 4.6 fertile tillers per plant that was higher than those obtained in somaclones.

DISCUSSION

In wheat, somaclonal variation has been reported for several agronomic and phenotypic traits, such as plant height, leaf size, pollen fertility, and tolerance to aluminum toxicity (Ahloowalia, 1982; Carver & Johnson, 1989; Cheng *et al.*, 1992; Symillides *et al.*, 1995). In our study, seedlings at the *in vitro* stage identified phenotypic variants concerning mainly albinism and leaf malformations.

Fig. 1: Phenotypic variation observed in seedlings during the *in vitro* culture

(a) Phenotypically normal green seedlings; (b) Calli regenerating plantlets with abnormal leaves; (c) calli regenerating green and albino plantlets; (d) Calli regenerating only albino plantlets; (e) Calli regenerating only chimeric plantlets; (f) Calli regenerating chimeric and green plantlets



After transfer of plantlets to soil and their follow up to maturity, we noted a few cases of sterility and abnormalities in spike morphology. Chlorophyll deficiency or albinism is a standard marker of variation in the cytoplasmic genome. This variation is common in androgenesis in durum wheat and is a major problem for the application of haplodiploidisation in breeding programs of this species (Ghaemi & Sarrafi, 1994). In somatic embryogenesis, conversely, few reports have mentioned regeneration of albinos (Maddock *et al.*, 1983; Bouiamrine *et al.*, 1999). In our culture conditions, the number of albino plantlets regenerated in Isly and Karim remained very low. The frequency of albino plant regeneration depends on several factors such as genotype (Yasmin *et al.*, 2009), explant age (Chang *et al.*, 2003) and medium composition (Dahleen & Bregitzer, 2002). Genetic control of albinism from somatic tissues in cereals was also reported by Bregitzer and Campbell (2001) and Wang *et al.* (2002). In androgenesis,

Fig. 2: Phenotypic variation observed during cultivation in field conditions

(a) and (b) Sterile and abnormal spike morphology; (c) morphologically abnormal plants with abnormal fertile spike; (d) Plants showing variations in the height and number of tillers

QTLs responsible of albinism have been mapped in wheat (Torp *et al.*, 2001), triticale (González *et al.*, 2005) and barley (Muñoz-Amatriaín *et al.*, 2008).

The variations observed in R0 plants for spike morphology and sterility are very rare. Variants for the same traits were already reported (Maddock & Semple, 1986). R1 plants, coming from R0 plants presenting variations in fertile spikes, did not show defects in their spikes. Indeed somaclonal variation can be either of gene type, and therefore persist over generations and are transmitted by crossing; or of epigenetic type, not inherited, often resulting from a change in gene expression originating from DNA methylation (Kaeppler *et al.*, 2000). Comparison of somaclones to parents for the studied agronomic traits depended on genotype. For two important agronomic traits, 100 grain weight and number of grains per spike, somaclones did not show better performance compared to parents in three varieties. On the contrary, some somaclones were better than parents for traits such as number of fertile tillers per plant, plant height or number of days to heading. According to Carver and Johnson (1989), obtaining somaclonal variants with superior crop yields compared to parents, is particularly difficult. The work of Cheng *et al.* (1992) on winter wheat somaclones showed that majority of agronomic characteristics in somaclones and their progeny were low as compared to parents. However, variants were selected having improved earliness. Our results have also recorded significant duration reductions in the number of days to heading in somaclones Karim (Kar-SOM1b, Kar-

SOM1c, Kar-SOM1d & Kar-SOM1e) of Sebou somaclone Seb-SOM1d and of three somaclones in Isly (Isly-SOM1a, Isly-SOM1b Isly-SOM1c) compared to parents for the three varieties studied. In bread wheat, Arun *et al.* (2007) were able to obtain somaclonal variants with an important earliness in heading and in maturity phases. Other studies have also reported reduction in number of days to heading in somaclones of wheat compared to parents as was reported earlier (Suenaga & Kakajima, 1993; Villareal *et al.*, 1999).

Number of fertile tillers per plant showed significant differences between the somaclones and their parents. However, only one variant (Sebou, Seb-SOM1b) showed a significant improvement compared to the parent. Danci *et al.* (2008) reported that none of the studied somaclonal and gametoclonal variants had shown superiority over parents for this character in bread wheat. The improvements obtained for this character remained unreliable because it is determined not only by genetic factors but also largely by environment and physiological state of the plant (Beveridge *et al.*, 2003). Significant differences between parents and somaclones were also observed for plant height in three varieties that has also been reported by several authors both in wheat and in other cereal species (Ahloowalia & Sherington, 1985; Symillides *et al.*, 1995; Raziuddin *et al.*, 2010).

The study concluded that many somaclones appeared to display superiority compared to the parent various. Further studies are needed for a complete evaluation of these variations for use in breeding programs. For proper evaluation of variation stability, the plants should be grown in different climatic conditions and the studied characters monitored for a long time.

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REFERENCES

Ahloowalia, B.S., 1982. Plant regeneration from callus culture in wheat. *Crop Sci*., 22: 405–410

Ahloowalia, B.S. and H. Sherington, 1985. Transmission of somaclonal variation in wheat. *Euphytica*, 3: 525–537

Arun, B., A.K. Joshi, R. Chand and B.D. Singh, 2003.Wheat somaclonal variants showing, earliness, improved spot blotch resistance and higher yield. *Euphytica*, 132: 235–241

Arun, B., B.D. Singh, S. Sharma, R. Paliwal and A.K. Joshi, 2007. Development of somaclonal variants of wheat (*Triticum aestivum* L.) for yield traits and disease resistance suitable for heat stressed and zero-till conditions. *Field Crops Res.*, 103: 62–69

Bairu, M.W., A.O. Aremu and J. Van Staden, 2011. Somaclonal variation in plants: causes and detection methods. *Plant Growth Regul.*, 63: 147–173

Beveridge, C.A., J.L. Weller, S.R. Singer and J.M.I. Hofer, 2003. Axillary meristem development. Budding relationships between networks controlling flowering, branching, and photoperiod responsiveness. *Plant Physiol.*, 131: 927–934

Brar, D.S. and S.M. Jain, 1998. Somaclonal variation: mechanism and application in crop improvement. *In*: Brar, D.S., S.M. Jain and B.S. Ahloowalia (eds.), *Somaclonal Variation and Induced Mutations in Crop Improvement*, pp: 15–37. Kluwer Academic Publications, London

Bregitzer, P. and R. Campbell, 2001.Genetic markers associated with green and albino plant regeneration from embryogenic barley callus. *Crop Sci.*, 41: 173–179

Breiman, A., D. Rotem-abarbanell, A. Karp and H. Shaskin, 1987. Heritable somaclonal variation in wild barley (*Hordeum spontaneum*). *Theor. Appl. Genet.*, 71: 637–643

Bouiamrine, E.H., K. Mzouri and M. Amssa, 1999. Effet du génotype et du milieu de culture dans la culture d'embryons immatures de blé dur (*Triticum durum* Desf.) et de blé tendre (*Triticum aestivum* L.) *In*: Aupelf-Uref (ed.), *Actualités Scientifiques, Biotechnologies, Amélioration Des Plantes et Securite Alimentaire*, p: 533. Estem, Paris, France

Carver, B.F. and B.B. Johnson, 1989. Partitioning of variation derived from tissue culture of winter wheat. *Theor. Appl. Genet*., 78: 405–410

Cassells, A.C. and R.F. Curry, 2001. Oxidative stress and physiological, epigenetic and genetic variability in plant tissue culture: implications for micropropagators and genetic engineers. *Plant Cell Tiss. Org. Cult.*, 64: 145–157

Chang, Y., J. Zitzewitz, P.M. Hayes and T.H.H. Chen, 2003. High frequency plant regeneration form immature embryos of elite barley cultivars (*Horedum vulgare* L. cv. Morex). *Plant Cell Rep.*, 21: 733–738

Cheng, X.Y., M.W. Gao, Z.Q. Liang, G.Z. Liu and T.C. Hu, 1992. Somaclonal variation in winter wheat: frequency, occurrence and inheritance. *Euphytica*, 64: 1–10

Dahleen, L.S. and P. Bregitzer, 2002. An improved media system for high regeneration rates from barley immature embryo-derived callus cultures of commercial cultivars. *Crop Sci.*, 42: 934–938

Danci, M., O. Danci and F. Berbentea, 2008. Researches concerning the variability of fertile tillers number for the somaclones and gametoclones of the wheat cultivar Dropia. *Rsearch J. Agric. Sci*., 40: 17–20

Dorffling, K. and G. Melz, 1997. Improvement of frost tolerance in winter wheat by in vitro selection of proline over producing mutants. *Acta Agric. Hung.*, 45: 295–299

Edallo, S., C. Zucchinali, M. Perenzin and F. Salimini, 1981. Chromosomal variation and frequency of spontaneous mutation associated with *in vitro* culture and plant regeneration in maize. *Maydica*, 26: 39–56

Evans, D.A. and W.R. Sharp, 1988. Somaclonal and gametoclonal variation. *In*: Evans, D.A., W.R. Sharp and P.V. Ammirato (eds.), *Handbook of Plant Cell Culture*, pp: 97–132. Macmillan Publishing Company, New York, USA

Evans, D.A., 1989. Somaclonal variation- Genetic basis and breeding applications. *Trends Genet*., 5: 46–50

Evans, D.A., W.R. Sharp and H.P. Medina-Filho, 1984. Somaclonal and gametoclonal variation. *American J. Bot.*, 71: 759–774

Ghaemi, M. and A. Sarrafi, 1994. The effect of the 'D' genome from synthetic wheat lines in anther culture response. *Plant Breed.*, 112: 76–79

González, J.M., L.M. Muñiz and N. Jouve, 2005. Mapping of QTLs for androgenetic response based on a molecular genetic map of × Triticosecale Wittmack. *Genome*, 48: 999–1009

Jain, S.M., 2001. Tissue culture-derived variation in crop improvement. *Euphytica*, 118: 153–166

Kabir, A.H., I. Mahfuz, M.A. Razvy, M.B. Ahmed and M.F. Alam, 2008. Indirect organogenesis and somaclonal variation in four rice cultivars of Bangladesh. *J. Appl. Sci. Res.*, 4: 451–458

Kaeppler, S.M., H.F. Kaeppler and Y. Rhee, 2000. Epigenetic aspects of somaclonal variation in plants. *Plant Mol. Biol.*, 43: 179–188

Karp, A., 1994. Origins, causes and uses of variation in plant tissue cultures. *In*: Vasil, I.K. and T.A. Thorpe (eds.), *Plant Cell and Tissue Culture*, pp: 139–151. Kluwer Academic Publications, Dordrecht, The Netherlands

Larkin, P.J. and W.R. Scowcroft, 1981. Somaclonal variation: a novel source of variability from cell cultures for plant improvement. *Theor. Appl. Genet.*, 60: 197–214

Larkin, P.J., S.A. Ryan, R.I.S. Brettell and W.R. Scowcroft, 1984. Heritable somaclonal variation in wheat. *Theor Appl. Genet*., 67: 443–456

Larkin, P.J. and W.R. Scowcroft, 1983. Somaclonal variation and crop improvement. *In*: Kosuge, T., C.P. Meredith and A. Hollaender (eds.), *Genetic Engineering of Plants*: *An Agricultural Perspective*, pp: 289–314. Plenum Press, New York

Lestari, E.G., 2006. *In vitro* selection and somaclonal variation for biotic and abiotic stress tolerance. *Biodiversitas*, 7: 297–301

Liang, H.S., H.B. Zheng and W.X. Li, 1996. The application of somaclonal variation in early maturity, high yield and high quality improvement in wheat. *J. North East Agric. University*, 3: 81–87

Li, X., X. Yu, N. Wang, Q. Feng, Z. Dong, L. Liu, J. Shen and B. Liu, 2007. Genetic and epigenetic instabilities induced by tissue culture in wild barley (*Hordeum brevisubulatum* (Trin.) Link). *Plant Cell Tiss. Org. Cult.*, 90: 153–168

Lörz, H., E. Göbel and P. Brown, 1988, Advances in tissue culture and progress towards genetic transformation of cereals. *Plant Breed*., 100: 1–25

Maddock, S.E. and J.T. Semple, 1986. Field assessment of somaclonal variation in wheat. *J. Exp. Bot.*, 37: 1065–1078

Maddock, S.E., V.A. Lancaster, R. Risiott and J. Franklin, 1983. Plant regeneration from cultured immature embryos and inflorescences of 25 cultivars of wheat (*Triticum aestivum* L.). *J. Exp. Bot.*, 34: 915–926

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant*, 15: 473–497

Muñoz-Amatriaín, M., A.M Castillo, X.W. Chen, L. Cistué and M.P. Vallés, 2008. Identification and validation of QTLs for green plant percentage in barley (*Hordeum vulgare* L.) anther culture. *Mol. Breed.*, 22: 119–129

R Development Core Team, 2012. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Raziuddin, J., Z.A. Bakht Swati, M. Shafi, U. Farhat and M. Akmal, 2010. Effect of cultivars and culture medium on callus formation and plant regeneration from mature embryos of wheat (*Triticum aestivum* L.). *Pakistan J. Bot.*, 42: 639–652

Suenaga, K. and K. Kakajima, 1993.Variation in doubled haploid plants of wheat obtained through wheat (*Triticum aestivum*) × maize (*Zea mays*) crosses. *Plant Breed*., 111: 120–124

Symillides, Y., Y. Henry and J. De Buyser, 1995. Analysis of Chinese spring regenerants obtained from short and long term wheat somatic embryogenesis. *Euphytica*, 82: 263–268

Torp, A.M., A.L. Hansen and S.B. Andersen, 2001. Chromosomal regions associated with green plant regeneration in wheat (*Triticum aestivum* L.) anther culture. *Euphytica*, 119: 377–387

Yasmin, S., I.A. Khan, A. Khatri, N. Seema, S.G. Nizamani and M.A. Arain, 2009. *In vitro* plant regeneration in bread wheat (*Triticum aestivum* L.). *Pakistan J. Bot.*, 41: 2869–2876

Wang, Z., D. Lehmann, J. Bell and A. Hopkins, 2002. Development of an efficient plant regeneration system for Russian wildrye (*Psathyrostachys juncea*). *Plant Cell Rep.*, 20: 797–801

Villareal, R.L., K.A. Mujeeb and P.J. Pena, 1999. Agronomic performance and quality characteristics of tissue culture derived lines of spring wheat (*Triticum aestivum* L.) cultivar Pavon. *Cereal Res. Com.*, 27: 41–48

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