

2, 4-D. The highest shooting percentages, ranging between 53 – 74% were recorded in MS medium supplemented with 2.0 mg/l 2, 4-D (Table 4). Significant differences ($p < 0.05$) in shoot induction frequency were detected at different 2, 4-D concentrations and among cultivars tested. The highest overall mean (48.5%) of shoot induction frequency was recorded for cultivar Argeen while the lowest (31.5%) was recorded for cultivar Bohain.

Embryogenic calli that initiated shoots after six weeks in 2.0 mg/L 2,4-D were transferred, separately, to MS media each containing a combination of 2.0 mg/L 2,4-D and one of four (0.5, 1.0, 1.5 and 2.0 mg/l) Zeatin concentrations. Root initiation and elongation was monitored and rooting percentages were recorded weekly for six weeks, for each shooted callus, in each of the MS-2, 4-D/Zeatin medium combination, results are shown in Plate 2 and Fig. 1. The highest regeneration percentage for each cultivar was recorded when 1.0 mg/l Zeatin was used. At this concentration, cultivar Sasraib showed significantly high regeneration percentage (49%) compared to the other cultivars tested. The highest regeneration percentages were recorded for cultivar Imam in Zeatin concentrations of 1.5

and 2.0 mg/l, while the lowest frequencies of 5, 6, 7 and 19% were recorded for cultivar Nasser in the four Zeatin concentrations used. It has been reported that cytokinins, such as Zeatin, 6-benzylaminopurine (BAP) and Kinetin when incorporated in the regeneration medium could improve shoot regeneration [22]; [5]. In this study, the addition of zeatin in the regeneration medium significantly improved regeneration capacity of callus which is consistent with the findings above.

The transfer of foreign genes into wheat by genetic engineering techniques requires the development of an efficient in vitro regeneration system. Results presented in this study are interesting because it may increase efficiency of gene transfer technology to genotypes that have low tissue culture response from immature embryos. An efficient regeneration system such as mature embryo culture may provide enough material for direct gene transfer studies. Therefore, mature embryos, which are readily available throughout the year, can be used as an effective explant source in wheat tissue culture.

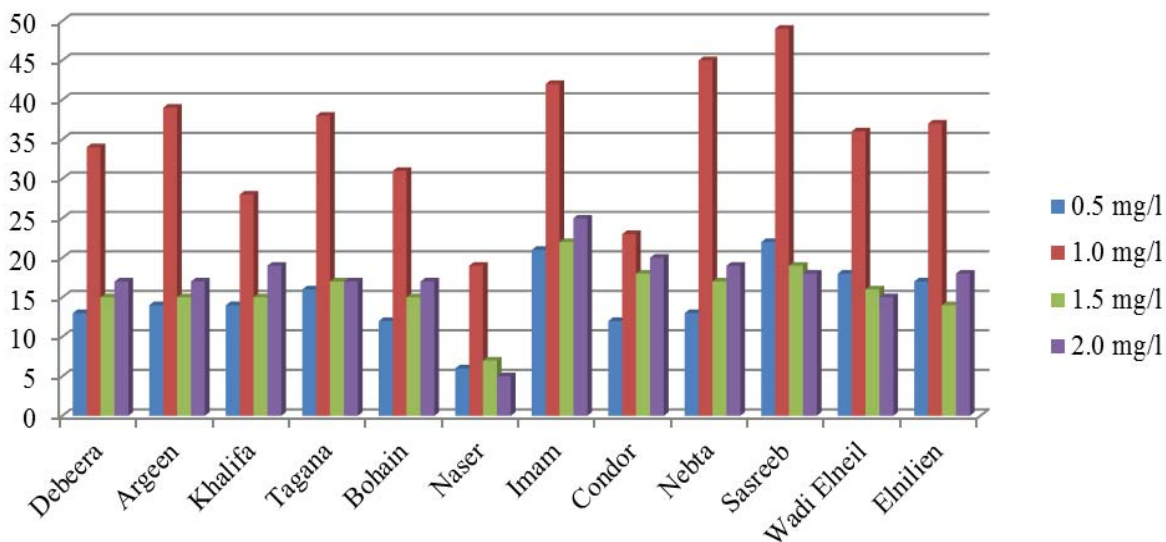


Figure 1: Regeneration of different wheat cultivars in MS medium supplemented with 2.0 mg/l 2, 4-D and 1.0 mg/l Zeatin

Table 1: Analysis of variance for callus and shoot induction frequencies of the 12 wheat cultivars in MS medium supplemented with different 2, 4-D concentrations.

Source of Variation	Callus induction frequency				Shoot induction frequency		
	df	SS	MS	F	SS	MS	F
Cultivars	11	296.949	26.995	10.659**	175.349	15.941	6.795**
PGRs Concentrations	5	811.074	162.215	64.052**	680.340	136.068	58.004**
Cultivars x PGRs Concentrations	55	79.376	1.443	0.570 ^{NS}	121.976	2.218	0.945 ^{NS}

**means are highly significant ($p < 0.05$), ^{NS} means are not significant



Plate 1: Callus induction and shoot initiation in MS medium supplemented with 2.0 mg/l 2, 4-D

Table 2: % callus induction of wheat cultivars in six 2,4-D concentrations

Cultivars	2,4-D concentration (mg/L)						Mean* ± SD
	0.5	1	1.5	2	3	4	
Debeera	5.6	4.7	5.4	7.7	5.2	4.8	5.57 ^b ± 0.21
Argeen	5.4	5.8	6.1	7.6	4.9	5.0	5.80 ^{bc} ± 0.21
Khalifa	4.4	5.7	6.7	7.9	5.7	4.9	5.88 ^{bc} ± 0.21
Taganna	4.5	5.3	6.1	8.0	5.0	4.8	5.62 ^b ± 0.21
Bohain	4.9	5.5	6.3	8.0	5.4	5.7	5.97 ^{bc} ± 0.21
Nasser	3.3	4.0	5.3	6.5	3.7	3.0	4.30 ^a ± 0.21
Imam	3.1	4.4	4.9	6.3	3.7	3.4	4.30 ^a ± 0.21
Gondor	4.4	5.0	5.9	7.9	6.0	5.1	5.72 ^{bc} ± 0.21
Nabta	5.3	6.1	6.7	8.2	5.8	6.0	6.35 ^c ± 0.21
Sasreeb	4.4	5.1	5.9	7.8	5.8	4.8	5.63 ^b ± 0.21
Wadi EL Niel	5.3	6.0	7.2	8.6	5.0	5.2	6.22 ^{bc} ± 0.21
Elnielien	4.9	5.8	6.7	8.2	5.0	4.9	5.9 ^{bc} ± 0.21
Mean* ± SD	4.7 ^a ± 0.15	5.3 ^c ± 0.15	6.1 ^d ± 0.15	7.8 ^e ± 0.15	5.1 ^{bc} ± 0.15	4.8 ^{ab} ± 0.15	5.63 ± 0.21

* means with different letters are significantly different at $p = 0.05$.

Table 3: Weight and colour of induced calli from mature embryos of different wheat cultivars in MS medium supplemented with different 2,4-D concentrations

Cultivars	2,4-D concentrations (mg/L)												Mean
	0.5		1		1.5		2		3		4		
	Weight (mg)	Colour	Weight (mg)	Colour	Weight (mg)	Colour	Weight (mg)	Colour	Weight (mg)	Colour	Weight (mg)	Colour	
Debeera	0.030	yellow	0.034	green	0.047	yellow	0.051	green	0.402	creamy	0.430	yellow	0.166
Argeen	0.245	white	0.255	creamy	0.026	white	0.027	white	0.350	yellow	0.400	green	0.217
Khalifa	0.135	brown	0.140	white	0.155	brown	0.167	yellow	0.492	white	0.516	brown	0.268
Tagana	0.023	yellow	0.033	yellow	0.035	white	0.044	creamy	0.363	brown	0.430	cream	0.155
Bohain	0.015	creamy	0.016	green	0.017	creamy	0.018	brown	0.340	white	0.350	brown	0.126
Nasser	0.011	green	0.012	creamy	0.013	green	0.013	green	0.330	yellow	0.340	creamy	0.120
Imam	0.019	brown	0.020	yellow	0.022	yellow	0.022	yellow	0.340	creamy	0.395	green	0.136
Condor	0.018	green	0.019	creamy	0.019	creamy	0.020	brown	0.375	yellow	0.380	white	0.138
Nebta	0.017	yellow	0.018	yellow	0.019	green	0.020	white	0.360	white	0.375	yellow	0.135
Sasreeb	0.045	creamy	0.056	creamy	0.057	yellow	0.058	creamy	0.435	creamy	0.510	creamy	0.193
Wadi El Niel	0.035	white	0.036	green	0.037	white	0.037	creamy	0.360	brown	0.410	yellow	0.152
Elnielien	0.010	brown	0.011	yellow	0.012	brown	0.013	brown	0.310	green	0.320	white	0.113
Mean	0.050		0.054		0.038		0.041		0.371		0.405		

Table 4: Shoot initiation frequency of different wheat cultivars at different 2,4-D concentrations

Cultivars	2,4-D Concentration (mg/L)						Mean* ± SD
	0.5	1.0	1.5	2.0	3.0	4.0	
Debeera	3.9	3.4	4.0	5.6	3.8	3.5	4.03 ^{abc} ± 0.20
Argeen	4.0	4.8	5.2	6.9	4.2	4.0	4.85 ^a ± 0.20
Khalifa	3.1	4.8	5.3	5.3	5.1	3.4	4.50 ^a ± 0.20
Taganna	2.8	4.0	4.6	6.2	3.8	2.6	4.0 ^a ± 18.0
Bohain	4.0	4.5	5.6	6.8	4.8	3.3	31.5 ^a ± 12.4
Nisir	2.7	3.2	4.1	5.5	2.8	2.3	25.3 ^a ± 19.2
Imam	2.6	3.6	3.8	5.6	2.8	3.1	44.7 ^a ± 14.2
Gondor	3.5	4.1	4.1	5.9	3.9	3.5	34.2 ^a ± 8.0
Nabta	2.8	3.9	5.4	6.5	5.0	4.8	36.3 ^a ± 18.7
Sasreeb	2.3	2.9	4.5	5.7	4.1	3.5	36.7 ^a ± 22.8
Wadi EL Niel	3.7	4.7	5.9	7.4	3.9	4.1	32.8 ^a ± 14.9
Elnielien	3.6	4.3	5.5	6.3	3.8	3.3	32.8 ^a ± 15.9
Mean* ± SD	3.3 ^a ± 0.14	4.0 ^b ± 0.14	4.8 ^c ± 0.14	6.1 ^a ± 0.14	4.0 ^b ± 0.14	3.5 ^a ± 0.14	33.9 ± 15.7

* means with different letters are significantly different at p = 0.05.



Plate 2: Regeneration in MS medium supplemented with 2.0 mg/l 2, 4-D plus 1.0 mg/l Zeatin.

References

- [1] Agricultural Statistics Department (ASD) report (2011). Ministry of Agriculture, Sudan, Agricultural Statistics Department, and the Proposed Work Programme for 2012-2013 Report on ECA's Statistical Activities in 2010-2011.
- [2] Ahmed, K., Bartók, T. and Sági, F. (1992). A modified method for rapid callus induction by utilization of endosperm metabolites in mature and immature seeds of bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* L.). *Cereal Res. Comm.* 20, 81–86.
- [3] Araus, J.L., Slafer, G.A., Reynolds, M.P., Royo, C. (2002). Plant breeding and drought in C3 cereals: what should we breed for?. *Ann. Bot.* 89: 925–940.
- [4] Ashraf, M., Qureshi, A. S. and Ghafoor, A. (2004). Total wheat DNA variation into varieties using known primers of the genes induced in dehydration and salinity stress. *Pakistan J. Biol. Sci.* 6: 437 - 440.
- [5] Barro, F., Martin, A., Lazzeri, P.A. and Barceló, P. (1999). Medium optimization for efficient embryogenesis and plant regeneration from immature inflorescences and immature scutella of elite cultivars of wheat, barley and tritordeum. *Euphytica* 108: 161-167.
- [6] Benderradji, L., Brini, F., Ben Amar, S. et al. (2012). Sodium transport in seedlings of two bread wheat (*Triticum aestivum* L.) genotypes differing in their tolerance to salt stress. *Australian J. Crop Sci.* 5(3): 233–241.
- [7] Benderradji, L., Brini, F., Kellou, K., Ykhlef, N., Djekoun, A., Masmoudi, K. and Bouzerzour, H. (2012). Callus induction, proliferation, and plantlets regeneration of two bread wheat (*Triticum aestivum* L.) genotypes under saline and heat stress conditions. *Int. Schol. Res. Net. Agronomy* 367851, 8p.
- [8] Brisibe, E.A., Gajdosava, A., Olesen, A. and Andersen, S. (2000). Cytodifferentiation and transformation of embryogenic callus lines derived from anther culture of wheat. *J. Exp. Bot.* 51: 187-196.
- [9] Chen, Q.J., Zhou, H.M., Chen, J. and Wang, X.C. (2006). A Gateway-based platform for multigene plant transformation. *Plant Mol. Biol.* 62: 927–936.
- [10] Curtis, B.C. (2002). Wheat in the World. In: Curtis, B.C., Rajaram, S. and Gómez, H. M. (eds). *Bread Wheat: Improvement and Production*. FAO Plant Production and Protection Series. 567P.
- [11] Delporte, F., Mostade, O. and Jacquemin, J.M. (2001). Plant regeneration through callus initiation from thin mature embryo fragments of wheat. *Plant Cell Tiss. Org. Cult.* 67: 73-80.
- [12] Dencic, S., Kastori, R., Kobiljski, B. and Duggan, B. (2000). Evaluation of grain yield and its components in wheat cultivars and landraces under near optimal and drought conditions. *Euphytica* 113: 43-52.
- [13] El-Meleigy, A., Gabr, M.F., Mohamed, F.H. and Ismail, M.A. (2004). Responses to NaCl salinity of tomato cultivated and breeding lines differing in salt tolerance in callus cultures. *Int. J. Agric. Biol.* 6 (1): 19–26.
- [14] Fahmy, A. H., El-Shafy, Y. H., El-Shihy, O. M. and Madkour, M.A. (2004). highly efficient regeneration system via somatic embryogenesis from immature embryos of Egyptian wheat cultivars (*Triticum aestivum* L.) using different growth regulators. *Arab J. Biotech.* 7(2): 229-238.
- [15] FAO, Food and Agriculture Organisation (2012). FAO/WFP Crop and Food Security Assessment Mission (CFSAM) -January 2011to Sudan, Special Report. 18 June 2011. Available from: <http://www.fao.org/docrep/010/ai469e/ai469e00.htm>.
- [16] Fennell, S., Bohorova, N., Van Ginkel, M., Crossa, J. and Hoisington, D. (1996). Plant regeneration from immature embryos of 48 elite CIMMYT bread wheats. *Theor. Appl. Genet.* 92: 163-169.
- [17] Gosch-Wackerle, G., Avivi, L. and Galun, E. (1979). Induction, culture and differentiation of callus from immature rachises, seeds and embryos of *Triticum*. *Zeitschrift für Pflanzenphysiologie* 91 (3): 267-278.
- [18] He, G.Y. and Lazzeri, P.A. (2001). Improvement of somatic embryogenesis and plant regeneration from

- durum wheat (*Triticum turgidum* var. durum Desf.) scutellum and inflorescence cultures. *Euphytica* 119: 369-376.
- [19] Irfan, H., Bushra, S., Hafeez, A.S., Riaz, A.K., Muhammad, Z.I. and Iqar, A.K. (2012). Establishment of efficient in vitro culture protocol for wheat land races of Pakistan. *African J. Biotechnol.* 11(11): 2782-2790.
- [20] Joyia, F.A. and Khan, M.S. (2013). Scutellum-derived callus-based efficient and reproducible regeneration system for elite varieties of indica rice in Pakistan. *Int. J. Agric. Biol.* 15: 27-33.
- [21] Kadri, A., Chalak, L., El Bitar, A., Nicolas, N., Mroué, S., Grenier, De. And March, G. (2014). In vitro plant regeneration system for two Middle East cultivars of chickpea (*Cicer arietinum* L.). *Adv. Crop Sci. Tech.* 2: 125. doi:10.4172/2329-8863.1000125.
- [22] Kereša, S., Bariša, M., Šarevič, H. and Gunjača, J. (2004). Influence of Zeatin on wheat regeneration from immature embryos. *Agric. Conspec. Sci.*, 69 (1): 17-20.
- [23] Ling, H-Q., Kriseleit, D. and Ganal, M. G. (1998). Effects of ticarcillin/potassium clavulanate on callus growth and shoot regeneration in Agrobacterium-mediated transformation of tomato (*Lycopersicon esculentum* Mill). *Plant Cell Rep.* 17: 843-847.
- [24] Machii, H., Mizuno, H., Hirabayashi, T., Li, H. and Hagio, T. (1998). Screening wheat genotypes for high callus induction and regeneration capability and immature embryo cultures. *Plant Cell Tiss. Org. Cult.* 53: 67-74.
- [25] Mahmood, K. (2011). Salinity tolerance in barley (*Hordeum vulgare* L.): effects of varying NaCl, K⁺/Na⁺ and NaHCO₃ levels on cultivars differing in tolerance. *Pakistan J. Bot.* 43(3): 1651-1654.
- [26] Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plantarum* 15: 473-497.
- [27] Özgen, M., Türet, M., Özcan, S. and Sancak, C. (1996). Callus induction and plant regeneration from immature and mature embryos of winter durum wheat genotypes. *Plant Breed.* 115(6): 455-458.
- [28] Özgen, M., Türet, M., Altnok, S. and Sancak, C. (1998). Efficient callus induction and plant regeneration from mature embryo culture of winter wheat (*Triticum aestivum* L.) Genotypes. *Plant Cell Rep.* 34(2): 331-335.
- [29] Przetakiewicz, A., Orczyk, W. and Nadolska-Orczyk, A. (2003). The effect of auxin on plant regeneration of wheat, barley and triticale. *Plant Cell Tiss. Org. Cult.* 73: 245-256.
- [30] Redway, F.A., Vasil, V., Lu, D. and Vasil, I.K. (1990). Identification of callus types for long term maintenance and regeneration from commercial cultivars of wheat (*Triticum aestivum* L.). *Theor. Appl. Gen.* 79: 609-617.
- [31] Sahrawat, K.L., Ravi Kumar, G. and Rao, J.K. (2002). Evaluation of triacid and dry ashing procedures for determining potassium, calcium, magnesium, iron, zinc, manganese, and copper in plant materials. *Commun. Soil Sci. Plant Anal.* 33 (1-2): 95-102.
- [32] Sarker, R.H. and Biswas, A. (2002). In vitro plantlet regeneration and Agrobacterium-mediated genetic transformation of wheat. *Plant Tiss. Cult.* 12(2): 155-165.
- [33] Sears, R.G. and Deckard, E. L. (1982): Tissue culture variability in wheat: callus induction and plant regeneration. *Crop Sci.* 22, 546-550.
- [34] Shah, M.I., Jabeen, M. and Hai, I. (2005). In vitro callus induction, its proliferation and regeneration in seed explants of wheat (*Triticum aestivum* L.). *VARLU-268. Pakistan J. Bot.* 35(2): 209-217.
- [35] Taji, A., Kumar, P.P. and Lakshmanan, P. (2002). *In vitro Plant Breeding*, Food Products Press, New York, 167 pp.
- [36] Viertel, K. and Hess, D. (1996). Shoot tips of wheat as an alternative source for regenerable embryogenic callus cultures. *Plant Cell Tiss. Org. Cult.* 44: 183-188.
- [37] Wu, B.H., Zheng, Y.L., Liu, D.C. and Zhou, Y.H. (2002). Trait correlation of immature embryo culture in bread wheat. *Plant Breed.* 121: 1-5.
- [38] Yasmin, R., Javed, F. and Arfan M. (2001). Somatic embryogenesis in callus culture of wheat (*Triticum aestivum* L.) accession 235/2. *Int. J. Agric. Biol.* 3(2): 163-166.
- [39] Yu, Y., Wang, J., Zhu, M.L. and Wei, Z.M. (2008). Optimization of mature embryo-based high frequency callus induction and plant regeneration from elite wheat cultivars grown in China. *Plant Breed.* 127 (3): 249-255.
- [40] Zamora, A.B. and Scoot, K.J. (1983). Callus formation and plant regeneration from wheat leaves. *Plant Sci. Let.* 29: 183-189.