African Journal of Biotechnology Vol. 10(70), pp. 15749-15755, 9 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1495
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Plant regeneration in wheat mature embryo culture

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Accepted 15 September, 2011

Success in genetic engineering of cereals depends on the callus formation and efficient plant regeneration system. Callus formation and plant regeneration of wheat mature embryos were assessed by using 12 different methods with 4 genotypes. Genotype significantly affected the formation of callus, embryogenic callus and plant regeneration. Only two methods (methods #2 and 9) produced plant regeneration. The highest plant regeneration for all genotypes with endosperm that supported mature embryos was observed in method #9 which contain MS medium supplemented with 12 mg/l dicamba + 0.5 mg/l IAA.

Key words: Callus formation, explant, genotype, plant growth regulators.

INTRODUCTION

Success in genetic engineering of cereals depends on the callus formation and efficient plant regeneration system. Transgenic wheat plants can be regenerated through *Agrobacterium* transformation (Haliloglu and Baenzinger, 2003) and biolistic bombardment (Patnaik and Khurana, 2003). The most common explant type used for wheat transformation is immature embryo. However, special measures are required to obtain imamture embryos and its use is restricted after a while. Therefore, mature embryos are significant alternative for callus and somatic embryo formation, which can be used in wheat transformation.

Efficient callus formation and plant regeneration depend on: genotype (Sears and Deckard, 1982; Mathies and Simpson, 1986; Fennel et al., 1996), explant source (Ozias-Akins and Vasil, 1982; Redway et al., 1990), growth conditions of donor plant (Hess and Carman, 1998) and culture medium (Mathias and Simpson, 1986; Elena and Ginzo, 1988; Fennel et al., 1996).

When mature embryos are used as explant source, frequency of plant regeneration is low as compared to immature embryo culture. In addition, non-endosperm supported mature embryos directly from seeds (Ozias-Akins and Vasil, 1983; Kato et al., 1991; Kintzios et al., 1996; Varshney et al., 1999; Mendoza and Kaeppler, 2002; Li et al., 2003; Patnaik and Khurana, 2003; Zale et al., 2004), thin pieces of mature embryos (Delporte et al., 2001) and endosperm supported mature embryos (Ozgen et al., 1996, 1998; Chen et al., 2006; Filippov et al., 2006) have been used for callus formation and plant regeneration.

The aim of this research was to determine a suitable regeneration method, which can be used in wheat mature embryo culture. Therefore, 12 different modified methods used previously in wheat mature embryo culture and 4 different wheat cultivars were used.

MATERIALS AND METHODS

Mature embryos of genotypes Kırik, Doğu 88, Bezostaja 1 and Kate A-1 were used as explant source. Mature embryos were employed as non-endosperm supported (all methods except method 9) and endosperm supported (method 9 only) based on the methods. Callus formation and plant regeneration of mature embryos was assessed using 12 different methods (Table 1) to determine the best method of regeneration. Methods used in this research were compiled by modification of methods previously mentioned in literature (Varshney et al., 1999; Mendoza and Kaepler, 2002; Li et

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Abbreviations: MS, Murashige and Skoog (1962) medium; **B5,** Gamborg et al. (1968) medium; **2,4-D,** 2,4-dichlorophenoxy acetic acit; **Dicamba,** 3,6-dichloro-2-methoxybenzoic acid; **Picloram,** 4-amino-3,5,6-trichloropicolinic acid; **IAA,** ındole-3-acetic acid; **NAA,** 1-naphthaleneacetic acid; **BAP,** 6-benzylaminopurine; **TDZ,** thidiazuron, 1-phenyl-3-[1,2,3 thiadozol-5-yl] urea.

Table 1. Composition of callus formation and plant regeneration medium based on the methods and callus culture periods.

Culture media	Ingredient	Method											
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Callus formation medium	MS Salts[1] (g/l)	4.43	4.43	4.43	4.43	4.43	4.43	4.43	4.43	4.43	4.43	4.43	4.43
	MS Vitamins (1000x)[2] (ml/l)	1	1	1	1	1	1	1	1	1	1	-	1
	B5 Vitamins (1000x)[2] (ml/l)	-	-	-	-	-	-	-	-	-	-	1	-
	Agar[1] (g/l)	-	7	-	-	-	-	-	-	7	-	7	7
	Phytagel[1] (g/l)	2	-	2	2	2	2	2	2	-	2.5	-	
	Sucrose[1] (g/l)	20	20	-	20	20	20	20	20	20	20	30	20
	Maltose[1] (g/l)	-	-	40	-	-	-	-	-	-	-	-	-
	2,4-D (mg/l)	2.5	8	0.5	-	-	-	-	2	-	-	-	-
	Dicamba (mg/l)	-	-	-	4	4	4	4	-	12	4	4	8
	Picloram (mg/l)	-	-	2.2	-	-	-	-	-	-	-	-	-
	IAA (mg/l)	-	-	-	-	-	-	-	-	0.5	-	-	-
	L-Glutamine[2] (g/l)	-	-	0.5	0.5	0.5	0.5	0.5	-	-	5	0.3	-
	Casein hydrolyzate[2] (g/l)	-	-	0.1	0.1	0.1	0.1	0.1	0.2	-	1	0.5	-
	Magnesium chloride[1] (g/l)	-	-	0.75	0.75	0.75	0.75	0.75	-	-	-	-	-
	MES hydrate[1] (g/l)	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95
	Ascorbic acid (50 mg/ml)[2]	-	-	2 ml	2 ml	2 ml	2 ml	2 ml	-	-	-	-	-
	Myo-inositol[2] (mg/l)	-	-	-	-	-	-	-	0.2	-	-	-	-
	Glycine[2] (mg/l)	-	-	-	-	-	-	-	-	-	-	2	-
	Culture period (Week)	4	4	4	2	3	2	3	3	3	4	4	4
Plant regeneration medium	MS Salts[1] (g/l)	4.43	4.43	4.43	4.43	4.43	4.43	4.43	4.43	4.43	4.43	4.43	4.43
	MS Vitamins (1000x)[2] (ml/l)	1	1	1	1	1	1	1	1	1	1	-	1
	B5 Vitamins (1000x)[2] (ml/l)	-	-	-	-	-	-	-	-	-	-	1	-
	Agar[1] (g/l)	-	7	-	-	-	-	-	-	7	-	7	7
	Phytagel[1] (g/l)	2	-	2	2	2	2	2	2	-	2.5	-	
	Sucrose[1] (g/l)	20	20	-	20	20	20	20	20	20	20	30	20
	Maltose[1] (g/l)	-	-	40	-	-	-	-	-	-	-	-	-
	Dicamba (mg/l)	-	-	-	0.01	0.01	-	-	-	-	0.01	-	-
	L-Glutamine[2] (mg/l)	-	-	-	-	-	-	-	-	-	5	0.3	-
	Casein hydrolyzate[2] (g/l)	-	-	-	-	-	-	-	0.2	-	1	0.5	-
	MES hydrate[1] (g/l)	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95
	Ascorbic acid (50 mg/ml)[2]	-	-	2 ml	2 ml	2 ml	2 ml	2 ml	-	-	-	-	-
	Myo-inositol[2] (g/l)	-	-	-	-	-	-	-	0.1	-	-	-	-
	Glycine[2] (mg/l)	-	-	-	-	-	-	-	-	-	-	2	
	BAP (mg/l)	-	-	-	0.5	0.5	-	-	0.5	-	0.5		
	TDZ (mg/l)	-	-	-	-	-	-	-	-	-	-	1	
	NAA (mg/l)	-	-	-	-	-	-	-	0.02	-	-	-	

[1]:Heat-labile compound; [2]:thermolabile compound.

al., 2003; Patnaik and Khurana, 2003; Haliloglu and Baenzinger, 2005; Filippov et al., 2006; Ahmet and Adak, 2007; Aydin, 2011).

Seeds were washed with tap water and surface-sterilized with 70% ethanol for 5 min, treated for 25 min with solution containing 1% sodium hypochlorite with a few drops of Tween 20 with constant stirring, and rinsed three times with sterile distilled water thereafter. The seeds were imbibed in sterile water for 16 to 17 h at 4°C in the dark. Mature embryos were aseptically dissected out and transferred to different callus initiation media with different time periods based on methods for callus formation and kept in the dark at 25°C. Then, calli were transferred to regeneration media based on methods (Table 1) for 30 days with a 16 h lights (light intensity of 62 µmol $m^{-2}s^{-1}$) and 8 h dark photoperiod at 25°C. All regenerated plantlets were transferred to MS medium supplemented with 20 g/l

sucrose and 7 g/l agar in Magenta boxes until they attained 10 to 12 cm plant height.

All media were adjusted to pH 5.8 with 1 N NaOH. Media solutions containing basal salts and solidifying agent were autoclaved at 121°C for 15 min for sterilization. Vitamins and plant growth regulators were filter-sterilized. Callus formation (%) and embryogenic callus formation (%) were determined prior to transfer of the calli to plant regeneration medium. Whereas, responded embryogenic callus and regeneration efficiency were determined after 30 days in regeneration medium.

This study was carried out in complete randomized experimental design of 4 × 12 factorial arrangements with 4 replicates. Each Petri dish was considered as replication and 20 mature embryos were cultured into each Petri dish. Effects of genotypes and methods on callus formation, embryogenic callus formation, and responded embryogenic callus formation and regeneration efficiency were investigated. Analysis of variance (ANOVA) was performed using General Linear Model (GLM) procedure in SAS (SAS İnstitute, 1995). Means treatments and interactions were compared using the Duncan's multiple range test.

RESULTS

Callus formation

Main effects of both genotypes ($p = 0.002$) and methods ($p = 0.001$) on callus formation were significant. The highest callus formation occurred in Bezostaja-1 with 98.5%. Whereas, the lowest callus formation was observed in genotype Kate A-1 with 96.7%. This percentage was determined as 98.4% in both genotypes Kırik and Doğu 88, and there was no significant difference between them. When methods were compared based on callus formation, the highest callus formation (100%) was observed in methods #6, 7, 9 and 10 and the lowest one (91.9%) was method #8 (Table 2).

The fact that callus formation of genotype showed differences based on methods, resulted in significant genotype x method interaction ($p = 0.001$). When genotypes were compared based on average callus formation percentage for each method, significant differences ($p<0.05$) were seen among genotypes in methods #2, 4 and 8 (Table 2). When methods were compared for callus formation based on genotypes, there were significant differences among methods in genotypes, except Doğu 88. The highest callus formation (100%) was observed in genotype Kırik in methods #2 (Figure 1A), 6, 7, 8, 9, 10, 11 and 12; in genotype Doğu 88 in methods #2, 4, 6, 7, 9 and 10; in genotypes Bezostaja-1 and Kate A-1 in methods #4, 5, 6, 7, 9 (Figure 1A) and 10.

Embryogenic callus formation

Calli were categorized as embryogenic callus (EC) and non-embryogenic callus (NC). EC was characterized by cream color, friable, compact in nature and contained embryogenic structure occurring separate or had fused nodules (Figure 1B). NE was white, loose and watery in nature. Embryogenic callus formation was strongly influenced by genotypes and methods ($p = 0.001$). Kate A-1 took the first position in terms of embryogenic callus formation with 92.2% and followed by Bezostaja-1 (91.8%), Kırik (88.9%) and Doğu 88 (88.0%) (Table 2). Kate A-1, Bezostaja-1 and Kırik, Doğu 88 were in the same group for this parameter (Table 2). On the other hand, when genotypes were compared with respect to methods, the highest average EC was observed in method #7 (97.8%) and the lowest EC in method #11 (77.5%) (Table 2). In addition, a significant interaction between genotypes and methods was observed ($p = 0.001$). There were significant differences among genotypes for methods #2, 3, 4, 5, 6, 8, 9, 10 and 12 based on EC formation (Table 2). On the other hand, when methods were compared for each genotypes, the highest EC formation was produced by genotype Kırik (100%) in method #9, genotype Doğu 88 in methods #4 and 6, genotype Bezostaja-1 in methods #4 and 5, genotype Kate A-1 in methods #4, 5, 6 and 7 (Table 2).

Responded embryogenic callus formation

Embryogenic calli that produced roots and shoots were considered as responded embryogenic callus (REC). While genotypes that did not have significant effect on REC ($p = 0.27$), were strongly influenced by methods ($p = 0.001$). Only somatic embryos in methods # 2 and 9 produced plant regeneration (Figure 1C), somatic embryos in other methods did not regenerate any plants, therefore, it was presented as 0.0%. The highest REC was determined in methods #9 (16.3%) and followed by method #2 with 5.3% (Table 2). When genotypes were compared in both methods, the highest REC was observed in method #9 (17.5%) with genotypes Kırik and Doğu 88 and in method #2 (10.0%) with genotype Kırik. These values in methods #9 and 2 for genotype Doğu 88 were 17.5 and 2.5%, for genotype Bezostaja-1, it was 15.0 and 5.0%, for Kate A-1 it was 15.0 and 3.8%, respectively.

Regeneration efficiency

Main effects of genotypes and methods were significant ($p = 0.01$). Genotype Kate A-1 (0.7 plant) was the highest in terms of average plant regeneration efficiency and Bezostaja-1 was the lowest (0.3 plant). This value was 0.6 plant for genotype Kırik (Table 2). Only somatic embryos of methods #2 and 9 had plant regeneration when methods were compared in terms of average plant regeneration efficiency (Figure 1D and E). Plants that were regenerated in other methods originated from base embryo. Therefore, plants that were regenerated in these methods were not taken into account. Significant differences was determined between methods #2 and 9

Table 2. Callus formation (%), embryogenic callus formation (%), responded embryogenic callus formation (%), regeneration efficiency based on methods and genotypes.

Parameter[1]	Method	Cultivars ($\overline{X} \mp S_{\overline{x}}$)				Mean
		Kırik	Doğu 88	Bezostaja-1	Kate A-1	
Callus formation (%)[a]	1	98.8±1.3^{AB}	96.3±1.3	98.8±1.3^{AB}	97.5±2.5^{AB}	97.8±0.8^{AB}
	2	100±0.0^{aA}	100±0.0^{a}	97.5±1.4^{abAB}	95.0±2.0^{bB}	98.1±0.8^{AB}
	3	96.3±1.3^{AB}	97.5±1.4	98.8±1.3^{AB}	97.5±2.5^{AB}	97.5±0.8^{B}
	4	91.3±3.2^{bC}	100±0.0^{a}	100±0.0^{aA}	100±0.0^{aA}	97.8±1.2^{AB}
	5	95.0±2.9^{BC}	97.5±1.4	100±0.0^{A}	100±0.0^{A}	98.1±0.9^{AB}
	6	100±0.0^{A}	100±0.0	100±0.0^{A}	100±0.0^{A}	100±0.0^{A}
	7	100±0.0^{A}	100±0.0	100±0.0^{A}	100±0.0^{A}	100±0.0^{A}
	8	100±0.0^{aA}	97.5±2.5^{a}	95.0±2.0^{aB}	75.0±0.0^{bC}	91.9±2.7^{C}
	9	100±0.0^{A}	100±0.0	100±0.0^{A}	100±0.0^{A}	100±0.0^{A}
	10	100±0.0^{A}	100±0.0	100±0.0^{A}	100±0.0^{A}	100±0.0^{A}
	11	100±0.0^{A}	95.0±2.9	95.0±2.9^{B}	98.8±1.3^{AB}	97.2±1.1^{B}
	12	100±0.0^{A}	97.5±2.5	97.5±1.4^{AB}	96.3±2.4^{AB}	97.8±0.9^{AB}
	Mean	98.4±0.5^{a}	98.4±0.5^{a}	98.5±0.4^{a}	96.7±1.0^{b}	
Embryogenic callus formation (%)	1	97.5±1.4^{AB}	90.0±4.6^{BC}	96.3±2.4^{A}	97.5±2.5^{A}	95.3±1.6^{B}
	2	96.3±1.3^{aAB}	85.0±4.1^{bC}	96.3±2.4^{aA}	95.0±2.0^{aA}	93.1±1.7^{B}
	3	90.0±2.0^{aBC}	90.0±2.0^{aBC}	87.5±2.5^{abBC}	81.3±2.4^{bB}	87.2±1.4^{C}
	4	85.0±5.4^{bCD}	100±0.0^{aA}	100±0.0^{aA}	100±0.0^{aA}	96.3±2.1^{AB}
	5	77.5±2.5^{bDE}	97.5±1.4^{aAB}	100±0.0^{aA}	100±0.0^{aA}	93.8±2.5^{AB}
	6	90.0±3.5^{bBC}	100±0.0^{aA}	95.0±2.0^{abAB}	100±0.0^{aA}	96.3±1.4^{AB}
	7	95.0±2.9^{AB}	98.8±1.3^{AB}	97.5±1.4^{A}	100±0.0^{A}	97.8±0.9^{A}
	8	91.3±1.3^{aBC}	91.3±4.3^{aBC}	85.0±4.6^{aCD}	67.5±1.4^{bC}	83.8±2.9^{CD}
	9	100±0.0^{aA}	90.0±0.0^{bBC}	95.0±2.9^{abAB}	95.0±2.9^{abA}	95.0±1.3^{AB}
	10	90.0±4.1^{aBC}	67.5±2.5^{bD}	77.5±4.8^{bD}	95.0±2.9^{aA}	82.5±3.2^{D}
	11	80.0±0.0^{DE}	72.5±4.3^{D}	77.5±1.4^{D}	80.0±4.3^{B}	77.5±2.0^{E}
	12	73.8±1.3^{bE}	73.8±1.3^{bD}	93.8±2.4^{aAB}	95.0±2.0^{aA}	84.1±2.8^{CD}
	Mean	88.9±1.3^{b}	88.0±1.7^{b}	91.8±1.3^{a}	92.2±1.6^{a}	
Responded embryogenic callus formation (%)[c]	1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0^{C}
	2	10±0.0	2.5±2.5	5.0±2.9	3.8±2.4	5.3±1.2^{B}
	3	00±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0^{C}
	4	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0^{C}
	5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0^{C}
	6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0^{C}
	7	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0^{C}
	8	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0^{C}
	9	17.5±2.5	17.5±2.5	15.0±2.9	15.0±2.9	16.3±1.3^{A}
	10	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0^{C}
	11	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0^{C}
	12	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0^{C}
	Mean	2.3±0.8	1.7±0.7	1.6±0.7	1.6±0.7	
Regeneration efficiency (number of plant)[d]	1	0.0±0.0^{C}	0.0±0.0^{B}	0.0±0.0^{B}	0.0±0.0^{C}	0.0±0.0^{C}
	2	2.9±0.2^{aB}	0.6±0.6^{bB}	0.6±0.6^{bB}	1.4±0.8^{abB}	1.4±0.4^{B}
	3	0.0±0.0^{C}	0.0±0.0^{B}	0.0±0.0^{B}	0.0±0.0^{C}	0.0±0.0^{C}
	4	0.0±0.0^{C}	0.0±0.0^{B}	0.0±0.0^{B}	0.0±0.0^{C}	0.0±0.0^{C}
	5	0.0±0.0^{C}	0.0±0.0^{B}	0.0±0.0^{B}	0.0±0.0^{C}	0.0±0.0^{C}
	6	0.0±0.0^{C}	0.0±0.0^{B}	0.0±0.0^{B}	0.0±0.0^{C}	0.0±0.0^{C}
	7	0.0±0.0^{C}	0.0±0.0^{B}	0.0±0.0^{B}	0.0±0.0^{C}	0.0±0.0^{C}

8	0.0±0.0^{C}	0.0±0.0^{B}	0.0±0.0^{B}	0.0±0.0^{C}	0.0±0.0^{C}
9	4.1±0.7^{bA}	3.3±0.3^{bA}	3.0±0.4^{bA}	6.5±0.5^{aA}	4.2±0.4^{A}
10	0.0±0.0^{C}	0.0±0.0^{B}	0.0±0.0^{B}	0.0±0.0^{C}	0.0±0.0^{C}
11	0.0±0.0^{C}	0.0±0.0^{B}	0.0±0.0^{B}	0.0±0.0^{C}	0.0±0.0^{C}
12	0.0±0.0^{C}	0.0±0.0^{B}	0.0±0.0^{B}	0.0±0.0^{C}	0.0±0.0^{C}
Mean	0.6±0.2^{a}	0.3±0.1^{b}	0.3±0.1^{b}	0.7±0.3^{a}	

[1]For each parameters, values with insignificant difference for each column and row are indicated with same capital letters and lowercase letters, respectively (p>0.05). [a](Callus number/Explants number) x 100; [b](Embryogenic callus number/Explants number) x 100; [c](Responded embryogenic callus number/Explants number) x 100; [d]Regenerated plant number / Responded embryogenic callus number

with respect to average plant regeneration efficiency, and plant regeneration efficiency of method #9 (4.2 plant) was higher than that of methods #2 (1.4 plant). A significant interaction between methods and genotypes was observed since regeneration efficiency was changed based on methods. Regeneration efficiency value of genotype Kate A-1 was 6.5 in method #9 and 1.4 in method #2. These values for genotype Kırik were 4.1 and 2.9, for genotype Doğu 88 were 3.3 and 0.6 and for genotype Bezostaja-1 3.0 and 0.6 in methods #9 and #2, respectively. When methods were compared with respect to genotypes, the highest regeneration efficiency was observed in method #9 (Table 2).

DISCUSSION

It is well known that success in *in vitro* callus formation and plant regeneration is highly dependent on genotype (Haliloglu and Baenziger, 2005). Significant genotype effect on callus formation, embryogenic callus formation, responded embryogenic callus formation and regeneration efficiency was observed in our study. Similar results were also reported by many researchers (Ozgen et al., 1996, 1998; Rashid et al., 2002; Sarker and Biswas, 2002; Li et al., 2003; Zale et al., 2004; Chen et al., 2006; Patnaik et al., 2006; Ahmet and Adak, 2007; Bi et al., 2007).

Filippov et al. (2006) generated 5.4 plant per explant by using Tayozhnaya genotype in his study. Whereas, 0.98 plant per explant were generated in Kate A-1 genotype by using same method which is method #9 that was the best medium in our study. Differences between two studies in terms of plant regeneration efficiency can be explained by different genotypes used for each study. Similarly, Bregitzer (1992) and Li et al. (2003) stated that the most influential factor on callus formation and plant regeneration in wheat was genotype. Likewise, Mathias and Simpson (1986) reported that genotype was more important than medium.

Method #9 which endosperm supported mature embryos was used and gave the highest plant regeneration among 12 different methods in this research. In the same way, endosperm supported mature embryos gave better regeneration efficiency than non-endosperm supported mature embryos in wheat (Bartok and Sagi, 1990; Chen et al., 2006), barley (He and Jia, 2008) and triticale (Birsin and Ozgen, 2004).

The reason why endosperm supported mature embryos gave better results can be explained by either the supply of more and readily available nutrition than artificial culture medium (Bartok and Sagi, 1990) or there could be some cells in embryos that can receive signal from endosperm and therefore, higher regeneration can be maintained (Chen et al., 2006). Although, all methods except method #2 which did not use endosperm supported approach produced embryogenic calli, there was no success in plant regeneration.

Type and amount of plant growth regulators play important role in plant tissue culture. Common auxin type of plant growth regulator used in wheat and other cereals is 2,4-D (Filippov et al., 2006). On the other hand, dicamba was determined as more effective than 2,4-D in researches and auxin types were compared in endosperm and non-endosperm supported studies in wheat (Mendoza and Kaeppler, 2002; Filippov et al., 2006). Papenfuss and Carmen (1987) reported that dicamba was used readily by metabolism and therefore, increase the shoot formation. However, 2,4-D proves to be more resistance to enzymatic digestion and reaction and, hence, stays in cells that are highly stable (Moore, 1989).

Plant regeneration was obtained in methods #2 (8 mg/l 2,4-D) and #9 (12 mg/l dicamba + 0,5 mg/l IAA). This results show that it is necessary to use high concentration of auxins in mature embryo culture. Filippov et al. (2006) stated that mature embryos contain more old cells and more differentiated tissues than immature embryos and this necessitate the use of high concentration of auxin for re-differentiation. They also reported that endosperm can absorb the plant growth regulators in endosperm supported mature embryo culture and therefore, it is necessary to use higher concentration of auxins in endosperm supported mature culture than in non-endosperm supported mature culture. On the other hand, Mendoza and Kaeppler (2002) obtained the best plant regeneration in medium containing 4 mg/l dicamba. Conversely, although same auxin type and doses were used in methods #4, 5, 6, 7 and 11 in the first round of our study, we were not able to regenerate any plants. This result can be as a result using different genotypes in our study and using model genotype “Bobwhite” in the other study.

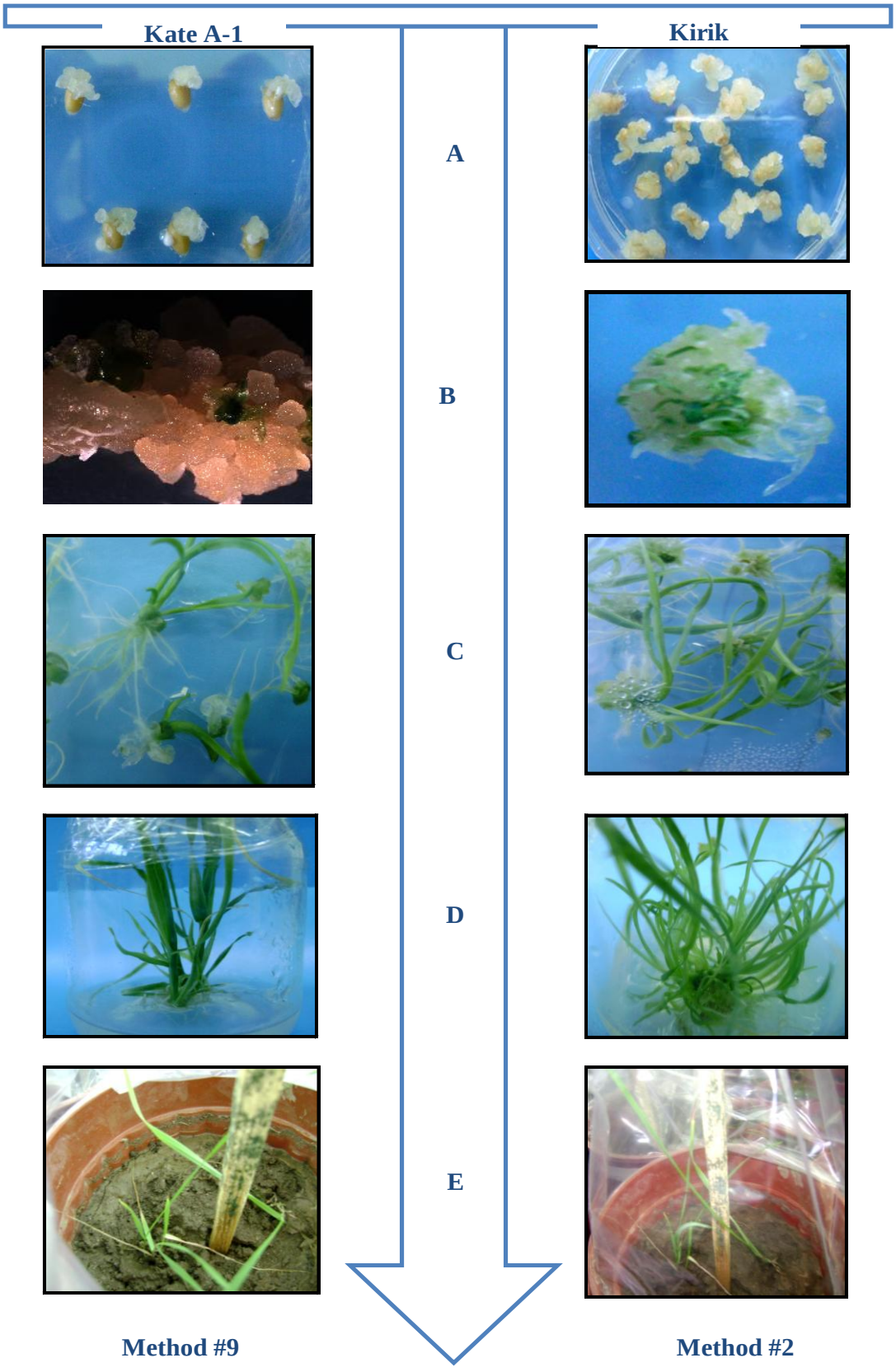

Figure 1. Calus formation and plant regeneration of wheat mature embryos of Kate A-1 and Kirik in methods #9 and 2, respectively. (A) callus formation (B) embryogenic callus, (C) plant regeneration, (D) plantlets in meganta boxes and (E) regenerated plant in soil.

Even thought methods #2 and 12 contained same medium and same amount (8 mg/l) of 2,4-D and dicamba, respectively except for non-endosperm supported mature embryos and hormones, there was no plant regeneration in method #12 in which dicamba was used as auxine. Filippov et al. (2006) obtained the best plant regeneration in 10 mg/l doses of 2,4-D and 12 mg/l of dicamba. These results show that higher concentration of dicamba is more effective as compared to 2,4-D. On the other hand, our results indicate the necessity of analyzing hormone type and doses factors, and they should not be combined and considered as one factor. Accordingly, Mendoza and Kaeppler (2002) showed that interaction of auxin type and doses were very important.

Consequently, success in wheat mature embryo culture highly depends on genotype, and endosperm supported mature embryos are more effective to plant regeneration as compared to non-endosperm supported mature embryo as explants.

ACKNOWLEDGEMENT

We are grateful for the financial support from the Scientific Research Projects Fund (BAP-2009/28) of Ataturk University, Erzurum, Turkey.

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