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Embryogenesis and plantlet regeneration optimization of wheat (*Triticum aestivum* L.)

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This study was conducted to develop an optimized embryogenesis and plantlet regeneration protocol for wheat (*Triticum aestivum* L.) cultivar Khaleefa. The effects of sucrose concentrations, activated charcoal, potassium phosphate, ammonium nitrate, potassium nitrate, chelated iron, different concentrations of benzyladenine (BAP), kinetin (KN) and 5-phenylcarbamoylamino (TDZ) in combination with indole-3-acetic acid (IAA) and different concentrations of silver nitrate were evaluated. Results showed that adding sucrose at (6%), activated charcoal (AC) at 0.5 g $^{-1}$ and the normal concentrations of potassium phosphate, ammonium nitrate, potassium nitrate and chelated iron in Murashige and Skoog basal medium (MS) salt were the best for promoting embryogenesis. Adding 2.0 mg $^{-1}$ BA in combination with 1.0 mg $^{-1}$ IAA gave the highest value of somatic embryos formation compared with other concentrations tested and addition of 20.0 mg $^{-1}$ silver nitrate to regeneration medium encouraged shoot regeneration.

Keywords: *Triticum aestivum*, embryogenesis, regeneration, mature embryo, MS medium.

Introduction

Application of biotechnological techniques has been advocated to circumvent problems associated with conventional breeding methods. The rapidly developed methods of molecular and genetic engineering provide powerful and novel means to supplement and complement the traditional methods of plant improvement (Kishore and Shewmaker, 1999; Sairam and Prakash, 2005). The transformation of all major cereals has now been achieved opening the way for the genetic engineering of new transgenic plants with modified agronomic traits, such as herbicide resistance, biotic and/or abiotic

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stresses resistance and grain quality and composition (Lazzeri and Shewry, 1994).

The lack of reproducible and dependable regeneration protocols suitable for different type of explants and genotype is a major hurdle towards an effective wheat functional genomics programme (Bhalla, 2006; Ganeshan *et al*., 2006; Vasil, 2007). If a suitable protocol for plant regeneration from mature embryogenic callus is available, research can be carried out on wheat transformation throughout the year. Considerable efforts are being made to improve wheat productivity by using biotechnology to meet that dramatic increase in cereal demand worldwide so a new approaches and technologies for generating new varieties are necessary.

Tissue culture techniques provide unique possibilities for overcoming the barriers of interspecific cross, asexual gene introgression, period of dormancy etc., also offers creation of variation through somaclonal and gametoclonal variations and facilitated rapid development of new varieties. Therefore, plant regeneration from callus cultures could provide useful germplasm for plant breeding program (Rahman *et al*., 2008).

Some treatments are known to promote somatic embryo maturation and germination such as osmotic treatment (Zhou *et al.*, 1991), low temperature (Hou *et al*., 1997), ethylene inhibitor AgNO3 (Purnhauser *et al*., 1987). Other components such as nitrogen source (Viertel and Hess, 1996), doubling the concentration of MS inorganic salts (Ozias-Akins and Vasil, 1983), carbon source and concentration (Eapen and Rao, 1985) have been evaluated. The positive effect of various medium growth regulator contents and combinations on somatic embryo induction and germination has been demonstrated (Ozias-Akins and Vasil, 1982; Husinger and Schauz, 1987; Fennell *et al*., 1996; Viertel and Hess, 1996).

The objective of this study was to develop a protocol for the formation of somatic embryos of wheat from mature zygotic embryos. This involved determination of the best nutrient medium components for embryogenic potential as well as for plantlet regeneration.

Materials and methods

Plant material, sterilization and medium

Mature dry seeds of *Triticum aestivum* (Khaleefa cultivar) were obtained from the Agricultural Research Corporation (ARC), Wheat Research Program-Wad Madani, Sudan.

Seeds were washed under running tap water for 30 minutes, and then socked in tap water for four to five hours, at room temperature, for enlarge size

and easy removed of embryos. Working in the laminar air flow hood, the soaked seeds were surface disinfested with (70%) ethanol for three minutes, then soaked in sodium hypochlorite (Clorox) 100% for twenty minutes, with continuously shaking, then washing several times with sterile distilled water. Embryos were separated from the embedded seeds using a sterile scalpel and forceps and placed in sterile glass bottles containing MS medium supplemented with 30 g $^{-1}$ sucrose, 2.0 mg $^{-1}$ 2, 4-D, solidified with 0.6% agar and the pH was adjusted to 5.8 and autoclaved at 121°C for 15 minutes.

Several treatments were conducted to promote embryogenesis and shoot regeneration. Calluses were cultured in MS medium supplemented with different concentrations of sucrose ranged from 0.0 to 120 g L^{-1}; the following concentrations (0.0, 0.25, 0.5, 1.0, 3.0 g $^{-1}$) of activated charcoal were investigated, potassium phosphate was testes in the following concentrations (0.0, 5, 10, 20, 30 ml^{-1}) , ammonium nitrate and potassium nitrate were tested in different concentrations ranged from 0.0 to 60 ml $^{-1}$; the effect of chelated iron was evaluated in several levels ranged from 0.0 to 30 ml $^{-1}$, effects of BAP, KN and TDZ singly and in combinations with IAA were investigated and the effect of silver nitrate in different concentrations ranged from 0.0 to 30 mg $^{-1}$ was also evaluated.

Experimental Design and Statistical Analysis

Experimental design was completely randomized. Data were statistically analyzed using ANOVA table on excel program and presented as average ± standard error. Means were separated according to Duncan's multiple range tests (Duncan, 1955) at 5% probability level.

Results and discussions

Effect of sucrose on embryogenesis and shoot regeneration

This experiment investigated the effect of sucrose at various concentrations (0.0 to 12%) on shoot formation. Results showed that the gradual increase in sucrose concentrations from 1.5 % to 6.0 % enhanced the formation of nodules (embryoids). Sucrose concentration at 6% in the medium gave the highest value of mean number of green nodules (0.8±0.3) and the highest percentage of nodulated callus (70%). The highest concentration of sucrose (12 %) tested inhibited the formation of green nodules and the percentage of nodulated callus (Table 1). No green nodules were formed in medium without sucrose (Table 1). Last and Brettell (1990) proposed that sucrose in culture medium functions both as a carbon source and an osmotic

regulator. It is rapidly hydrolyzed to glucose and fructose, nearly doubling the osmolarity of the medium. Both functions are critical for callus formation and shoot regeneration and could be detrimental to shoot regeneration in wheat Navarro-Alvarez *et al*. (1994). These results are consistent with the findings of Rasco-Gaunt *et al*. (2001) who obtained an increase in the percentage of callus formation by culturing immature embryos of wheat (cv. Florida) from 78 to 98% by increasing the sucrose concentration of culture medium from 3 to 6 or 9%.

Effect of activated charcoal (AC) on embryogenesis and shoot regeneration

To examine the effect of activated charcoal (A/C) on shoot regeneration, different levels of (A/C) in the range of 0.0 – 3.0 g^{-l} were added to the regeneration medium. A/C promoted the percentage of nodulated callus and the number of green nodules. The greatest number of green nodules (7.2±0.3) was produced in media containing 0.5 g/l A/C with highly significant differences compared to other treatments (Table 2, Fig 1). Furthermore, the percentage of nodulated callus was significantly enhanced by the addition of A/C with no difference for the A/C levels examined. However, levels of A/C > 0.5 g^{-l} inhibited the formation of green nodules and stimulated the appearance of root-like structures on the surface of the callus (Table 2). Medium without activated charcoal gave the least mean number of green nodules (0.8±0.2) and a percentage of nodulated calluses of 30 % (Table 2). The beneficial effects of activated charcoal may be attributed to removal of inhibitory substances present in the medium or originating from the cultured tissue (Johansson *et al*., 1982); establishing a degree of darkness during *in vitro* culture (Pan and Van Staden, 1998); adsorption of undesirable/inhibitory substances and adsorption of growth regulators and other organic compounds (Ebert *et al.*1993; Fridborg *et al.*1978). Our results, however, contrasted with those of Lashermes, (1992) which showed negative effects of A/C on anther culture in wheat attributing that to the binding ability of charcoal to inhibitory as well as enhancing substances in the medium.

Effect of potassium phosphate on embryogenesis and shoot regeneration

The effects of different concentrations of potassium phosphate were studied in order to determine the optimal level of this mineral element in the regeneration medium. The experiment showed that phosphates are required for shoot regeneration via organogenesis and/or embryogenesis; it promoted the formation of green nodules at 10 ml/l but not at either higher or lower concentrations. The greatest number of green nodules (4.2±0.2) was produced

on medium containing 10 ml^{-1} potassium phosphate (Fig1), a lesser number (3.7±0.3) on medium containing 5.0 ml^{-1} and the least (3.0±0.2) on medium containing potassium phosphate free medium. Increasing the concentration to 20.0 ml^{-1} or to 30.0 ml^{-1} potassium phosphate in the regeneration medium decreased the mean number of green nodules to 3.5±0.2 and 3.3±0.1 respectively. The values for percentage of nodulated callus were high on media containing 10.0 ml^{-1}, 5.0 ml^{-1} or potassium phosphate free medium, while they were reduced on media containing higher concentrations of potassium phosphate (Table 3). Results showed that formation of green nodules was significantly dependent on the concentration of phosphates of the nutrient media. Murashige (1974) advocated increasing the concentration of phosphates, higher than normal, in his famous MS medium by adding 170 mg^{-1} sodium phosphate. Supplement of additional phosphate to MS medium has been found beneficial for the growth and development of cultured plant tissues of some ornamental plants Miller and Murashige (1976) and pineapple (Idris *et al.*, 2006). A progressive decrease in values of all parameters measured was observed with increasing the concentration of phosphate in the nutrient medium of papaya and best growth and development were obtained on medium devoid of phosphate (Saadalla, 2007). Discrepancy in results could be attributed to differences in plant genotype, composition of the nutrient medium and incubation conditions. The requirements of mineral salts for *in vitro* growth and development of plant tissues are apparently dependent on species, cultivar and even tissue from the same species or cultivar (Maes *et al*., 1996; He *et al.*, 1989).

Effect of ammonium nitrate on embryogenesis and shoot regeneration

The effects of various concentrations of ammonium nitrate on shoot regeneration of mature embryo-derived callus of wheat were also studied. The normal concentration of this salt used in MS medium (20 mg^{-1}) was optimal for green nodules formation giving the highest value of mean number of green nodules 4.0±0.2 (Table 4, Fig 1), followed by 10 mg^{-1} with a mean number of 3.9±0.2 with no significant difference between them. Increasing the concentration of ammonium nitrate > 20 mg^{-1} decreased the formation of green nodules from 3.4±0.2 to 1.9±0.2 and percentage of nodulated callus from 80 % to 50 % (Table 4). High concentration of NH_4 might be harmful to plants tissues because of toxicity (Mengel and Kirkby, 1982) probably resulting from an alteration in nitrate: ammonia ratio. A balanced nitrate: ammonium ratio is vital for a better regeneration process (Nuutila *et al*., 2000; Chauhan and Kothari, 2004). In contrast to our results; Greer *et al*. (2009) reported that modification of the ammonium nitrate content in the direct somatic

embryogenesis induction medium can increase the number of primary embryos produced over two-fold in the elite hard red wheat cultivar "Superb". Menke-Milczarek and Zimny (2001) failed to establish a strong link between ammonium to nitrate ratio or their total concentration and the efficiency of somatic embryogenesis in wheat. They found that both ammonium and nitrate were necessary for the regeneration of the wheat cultivar "Grana".

Effect of potassium nitrate on embryogenesis and shoot regeneration

In this test we found that addition of potassium nitrate to the regeneration medium has a positive effect on embryoids formation. The data portrayed in Table 5 showed that the gradual increase in concentration of potassium nitrate in the regeneration medium up to 20.0 mg^{-l} significantly promoted the mean number of green nodules. Medium supplemented with 20 mg^{-l} potassium nitrate was most effective in inducing the regeneration of shoots from *in vitro* cultured wheat callus and the percentage of nodulated callus (Fig 1). High concentrations of potassium nitrate (> 20 mg^{-l}) have a negative effect on formation of green nodules and on percentage of nodulated callus (Table 5). These results are in accordance with those reported by (Feng and Ouyang, 1988) who noted that increases in the concentration of KNO_3 up to 15 mM resulted in a significant increase in the frequency of callus induction. Concentrations higher than 20 mM significantly decreased the frequency of callus induction in all tested cultivars. Additionally, significant increase in the regeneration frequency of green plantlet, and the ratio of green to albino regenerants increased significantly with increases in the concentration of KNO_3. These authors attributed the decrease in frequency of callus induction to the high concentration of the NO_3^- ion alone, while the effect of KNO_3 on green plantlet regeneration may be caused by both K^+ and NO_3^- ions, and that the effects of NO_3^- concentration were independent of NH_4^+ concentration in the medium.

Effect of chelated iron on embryogenesis and shoot regeneration

Various concentrations of chelated iron were tested to determine its effect on embryogenesis in wheat callus. The inclusion of 10 or 5 mg^{-l} chelated iron to the regeneration medium gave high mean number of green nodules (4.6±0.2) with no significant differences between them, higher additions (20 and 30 mg^{-l}) were inhibitory (Table 6).

These results concur with an earlier report (Novotny *et al*., 2000) with regard to the importance of a suitable form of iron supplement in the induction medium and for further development of induced somatic embryos in barley and

wheat genotypes, especially those providing few green plants via *in vitro* androgenesis, the same authors also found that genotypes capable of regenerating a great number of green plants were unresponsive to the lack of iron in the callus induction medium. Although chelated iron was found to be a suitable form of iron in the induction medium, androgenesis was also induced on media containing non-chelated iron (Fe^{2+} and Fe^{3+} ions) due to the use of cultivars or genotypes that are known to regenerate high numbers of green plants in anther-derived callus and are less sensitive to the lack of chelated iron in the induction medium and that embryos induced on iron free media do not lose its regeneration ability.

Effect of BAP, KN and TDZ singly and in combination with IAA on embryogenesis and shoot regeneration

The experiment investigated the effect of BAP, KN and TDZ singly and in combination with IAA at 1.0 mg^{-l} showed that the magnitude of response varied with type and concentration of the growth regulator(s) added to the culture medium. Embryogenesis occurred in all concentrations of the growth regulators tested, with significant statistical differences in mean number of green nodules. The greatest value of mean number of green nodules (6.6±0.1) was obtained with the combination treatment of 2.0 mg^{-l} BA and 1.0 mg^{-l} IAA; treatment 1.0 mg^{-l} BA and 1.0 mg^{-l} IAA came second with a 6.1±0.2 mean number of green nodules, followed by TDZ at 2.0 and 3.0 mg^{-l} in combination with 1.0 mg^{-l} IAA with a mean number of green nodules of 5.9±0.2. KN was the least effective cytokinin for the formation of green nodules compared to BA and TDZ (Table 7).

Our data concurs with those of Shah *et al*., (2003) who found that the regeneration of wheat plantlets was highest on MS medium supplemented with 2.0 mg /l BAP in combination with 1.0 mg^{-l} IAA. In general, the results of this study also agree with those of Lu (1993) and Mohmand, (1994) who obtained best results with MS medium containing 2.0 mg^{-l} BAP in combination with 1.0 mg^{-l} IAA and 1.0 mg^{-l} BAP in combination with 0.5 $mg^{/l}$ 2, 4-D.

The addition of BA during subculture greatly promoted embryogenic callus formation in wheat mature embryos culture Yu *et al.* (2008). The results of Tian *et al*. (1994) however, revealed that inclusion of TDZ alone in the subculture medium favored plant regeneration more than TDZ combined with the auxins. The failure of Kin to induce regeneration of wheat shoots from callus cultures over a wide range of concentrations has been also reported (Anju *et al*., 2003). Variations in results may be due to differences in cultivar, media composition, explant sources or incubation conditions. The promoted effects of exogenously applied IAA on somatic embryogenesis have been attributed to

low levels of endogenous IAA in the callus tissues (Filippov *et al*., 2006); this finding may explain the increase in the rate of embryogenic callus formation from mature embryos by the exogenously applied IAA.

Effect of silver nitrate on embryogenesis and shoot regeneration

Several concentrations of silver nitrate were examined for their potentiality to induce somatic embryogenesis and subsequent plant regeneration. The results presented in Table 8 summarize the effects of silver nitrate on wheat callus cultured *in vitro.* The values of the two parameters measured gradually increased with increasing silver nitrate concentration. The highest mean number (5.2±0.5) of green nodules was obtained on medium containing the highest two concentrations (25.0 and 30.0 mg^{-l}) tested. Regeneration of shoots occurred only on medium containing 20.0 mg^{-l} silver nitrate (Fig 1) with a regeneration percentage of 10% (Table 8).

The beneficial effects of silver nitrate on wheat tissue culture have been reported (Purnhauser *et al*., 1987 and Rasco-Gaunt *et al*., 1999); inclusion of AgNO3 in the medium was found to enhance shoot regeneration remarkably. The present data confirm the results of Racz *et al.* (1993) who cultured mature embryos of winter wheat on MSB medium supplemented with 20.0 mg^{-l} $AgNO_3$ for maximum and fast plantlets regeneration. The inclusion of silver nitrate in the culture medium effectively increased shoot formation in wheat, maize and rice tissue cultures (Purnhauser *et al*., 1987; Songstad *et al*., 1988; Lentini *et al.*, 1995).

Beyer (1976) and Lieberman (1979) attributed the beneficial effects of silver nitrate to its antagonistic action with ethylene rather than affecting ethylene biosynthesis; thus ameolrating the inhibitory effects of its accumulation in the headspace of the culture vessel.

Table 1. Effect of sucrose concentrations on shoot regeneration after 4 weeks of culture

Sucrose con. (g^{-1})	Noduated callus (%)	No. green nodules (Mean±SE)	Color and type of callus
0.0	0.0	0.0±0.0 ^{d}	Compact yellow callus
15.0	10	0.1±0.1 ^{d}	Compact yellow callus
30.0	30	0.5±0.2 ^{b}	Compact yellow callus
60.0	70	0.8±0.3 ^{a}	Compact green callus
120.0	50	0.3±0.1 ^{c}	Compact pale green callus

Means with the same letter (s) in the same column are not significantly different at 5% using Duncan Multiple Range Test.

Table 2. Effect of different levels of activated charcoal on shoot regeneration of wheat after 4 weeks of culture

Activated charcoal con. (g^{-1})	Noduated callus (%)	No.green nodules (Mean±SE)	Color and type of callus
0.0	30	0.8±0.2 ^{e}	Compact pale green callus with roots
0.25	100	3.2±0.1 ^{d}	Compact green callus with roots
0.5	100	7.2±0.3 ^{a}	Compact green callus with roots
1.0	100	4.8±0.1 ^{b}	Compact pale green callus with roots
3.0	100	4.5±0.1 ^{c}	Compact pale green callus with roots

Means with the same letter (s) in the same column are not significantly different at 5% using Duncan Multiple Range Test.

Table 3. Effect of potassium phosphate (KH_2PO_4) on shoot regeneration of wheat after 4 weeks of culture

Potassium phosphate con.(ml^{-1})	Noduated callus (%)	No.green nodules (Mean±SE)	Color and type of callus
0.0	100	3.0±0.2 ^{e}	Compact green callus with roots
5.0	100	3.7±0.3 ^{b}	Compact green callus with roots
10.0	100	4.2±0.2 ^{a}	Compact green callus with roots
20.0	90	3.5±0.2 ^{c}	Compact green callus with roots
30.0	80	3.3±0.1 ^{d}	Compact pale green callus with roots

Means with the same letter (s) in the same column are not significantly different at 5% using Duncan Multiple Range Test.

Table 4. Effect of ammonium nitrate (NH_4NO_3) on shoot regeneration of wheat after 4 weeks of culture

Ammonium nitrate con. (ml^{-1})	Noduated callus (%)	No.green nodules (Mean±SE)	Color and type of callus
0.0	100	2.3±0.2 ^{c}	Compact green callus with roots
10.0	100	3.9±0.2 ^{a}	Compact green callus with roots
20.0	100	4.0±0.2 ^{a}	Compact green callus with roots
40.0	80	3.4±0.2 ^{b}	Compact pale green callus with roots
60.0	50	1.9±0.2 ^{c}	Compact yellow callus with roots

Means with the same letter (s) in the same column are not significantly different at 5% using Duncan Multiple Range Test.

Table 5. Effect of potassium nitrate (KNO_3) on shoot regeneration of wheat after 4 weeks of culture

Potassium nitrate con. (ml $^{-1}$)	Noduated callus (%)	No. green nodules (Mean±SE)	Color and type of callus
0.0	100	3.9±0.2 ^{c}	Compact green callus with roots
10.0	100	4.3±0.2 ^{b}	Compact green callus with roots
20.0	100	4.4±0.2 ^{a}	Compact green callus with roots
40.0	60	3.3±0.2 ^{d}	Compact pale green callus
60.0	60	2.2±0.2 ^{e}	Compact pale green Callus

Means with the same letter (s) in the same column are not significantly different at 5% using Duncan Multiple Range Test.

Table 6. Effect of chelated iron on shoot regeneration of wheat after 4 weeks of culture

Chelated iron con. (ml $^{-1}$)	Noduated callus (%)	No. green nodules (Mean±SE)	Color and type of callus
0.0	100	4.4±0.3 ^{b}	Compact green callus with roots
5.0	100	4.6±0.2 ^{a}	Compact green callus with roots
10.0	100	4.6±0.2 ^{a}	Compact green callus with roots
20.0	85	3.3±0.1 ^{c}	Compact yellow callus
30.0	83	3.0±0.2 ^{d}	Compact Yellow callus

Means with the same letter (s) in the same column are not significantly different at 5% using Duncan Multiple Range Test.

Table 7. Effect of BAP, KN and TDZ in combination with IAA on shoots regeneration of wheat after 4 weeks of culture

Type of cytokinin	Con. (mg $^{-1}$)	IAA Con. (mg $^{-1}$)	Noduated callus (%)	No. green nodules (Mean±SE)
	0.0	1.0	100	4.5±0.1 ^{f}
BAP	1.0	1.0	100	6.1±0.2 ^{b}
	2.0	1.0	100	6.6±0.1 ^{a}
	3.0	1.0	100	5.4±0.3 ^{d}
	4.0	1.0	100	5.0±0.2 ^{e}
	1.0	1.0	100	3.0±0.2 ^{i}
KN	2.0	1.0	100	3.4±0.2 ^{h}
	3.0	1.0	100	3.9±0.2 ^{g}
	4.0	1.0	100	3.0±0.2 ^{i}
	1.0	1.0	100	5.4±0.3 ^{d}
TDZ	2.0	1.0	100	5.9±0.2 ^{c}
	3.0	1.0	100	5.9±0.2 ^{c}
	4.0	1.0	100	5.0±0.1 ^{e}

Means with the same letter (s) in the same column are not significantly different at 5% using Duncan Multiple Range Test.

Table 8. Effect of silver nitrate on shoot regeneration of wheat after 4 weeks of culture

Silver nitrate con. (mg^{-1})	Noduated callus (%)	No. green nodules (Mean±SE)	No. regenerated shoots	Regeneration (%)
0.0	30	0.5±0.2 ^{f}	0.0	0.0
5.0	0	1.1±0.3 ^{e}	0.0	0.0
10.0	80	2.5±0.4 ^{d}	0.0	0.0
15.0	80	3.0±0.5 ^{c}	0.0	0.0
20.0	85	3.8±0.5 ^{b}	1.0	10.0
25.0	85	5.2±0.5 ^{a}	0.0	0.0
30.0	85	5.2±0.5 ^{a}	0.0	0.0

Means with the same letter (s) in the same column are not significantly different at 5% using Duncan Multiple Range Test.

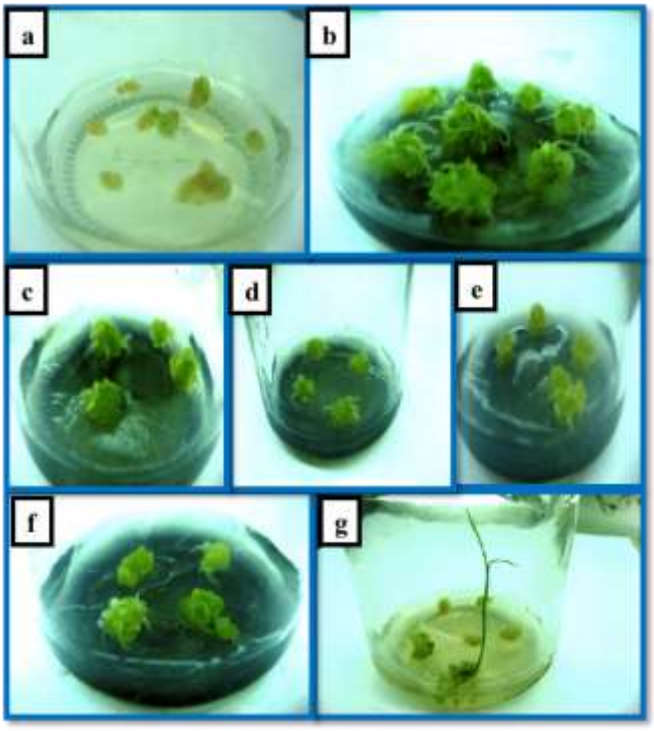

Fig. 1. Embryogenesis and regeneration in Khaleefa wheat cultivar: (a) Callus cultured on MS medium supplemented with 60.0 g^{-l} sucrose (b) Callus cultured in MS medium supplemented with 0.5 g^{-l} activated charcoal (c) Callus cultured in MS medium supplemented with 10.0 ml^{-l} potassium phosphate (d) Callus cultured in MS medium supplemented with 20.0 ml^{-l} ammonium nitrate (e) Callus cultured in MS medium supplemented with 20.0 ml^{-l} potassium nitrate (f) Callus cultured on MS medium supplemented with 10.0 ml^{-l} chelated iron (g) Plant let regeneration from callus cultured in MS medium supplemented with 20.0 mg^{-l} silver nitrate.

Conclusion

This study was conducted with the objective of optimizing embryogenesis and plantlet regeneration in *Triticum aestivum* (khaleefa Sudanese wheat cultivar) using several organic and inorganic substances known as promoting somatic embryos induction and maturation such as ethylene inhibitors, nitrogen sources, carbon sources, various growth regulators. Results demonstrated that increasing sucrose level up to (6%) and adding activated charcoal (AC) at 0.5 g^{-1} promoted embryogenesis. Inclusion of potassium phosphate, ammonium nitrate, potassium nitrate and chelated iron in the regeneration medium at the normal concentration encourage embryogenesis. Adding 2.0 mg^{-1} BA in combination with 1.0 mg^{-1} IAA gave the highest value of green nodules formation and addition of 20.0 mg^{-1} silver nitrate to regeneration medium encouraged shoot regeneration. Plants regenerated from mature embryos callus cultures could provide useful germplasm for wheat breeding programs and research can be carried out throughout the year.

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Abbreviations

BAP: benzyladenine
KN: kinetin
TDZ: 5-phenylcarbamoylamino
IAA: indole-3-acetic acid
AC: activated charcoal
MS: Murashige and Skoog basal medium

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