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Effect of Different Hormonal Combinations on Regeneration of Callus of *Gomphrena globosa* L.

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Abstract: The main aim of this study was to observe the effect of different hormonal combinations on regeneration of callus of *Gomphrena globosa* L. For this purpose callus was obtained from seeds *G. globosa* inoculated on MS medium supplemented with 4 mg L^{-1} 2, 4-D and 10% coconut milk. After callus formation callus was inoculated on Murashige and Skoog's medium supplemented with different combinations of BAP, NAA and GA_3 to observe different responses such as regeneration, callus friability, callus proliferation and pigmentation. In BAP and NAA root regeneration was observed at 1.5 mg L^{-1} BAP+1 mg L^{-1} NAA whereas rest of the combinations showed callus proliferation. In BAP and GA_3, root regeneration was observed in most of the combinations and some combinations also showed shoot induction. Shoot regeneration was observed on 0.5 mg L^{-1} BAP + 7 mg L^{-1} GA_3 and 1 mg L^{-1} BAP + 0.2 mg L^{-1} GA_3. The effect of all these combinations on auxin, acid phosphatase and soluble protein content was also observed.

Key words: *Gomphrena*, coconut milk, callus proliferation, pigmentation, root induction, auxin

INTRODUCTION

Gomphrena globosa L. (Amaranthacae) a commercial ornamental and medicinal plant is known for its colorful leaves and inflorescence. It is easily grown in warm climate and exhibit drought resistance. The genus *Gomphrena* comprises of approximately 120 species that are employed in the treatment of bronchial affections, diarrhea and fever. Different species of this genus also showed antibacterial, antimalarial and diuretic activities (Vieira *et al*., 1994; Moura *et al*., 2004). Besides as an ornamental plant, *G. globosa* is also commonly used for the treatment of diabetes, jaundice, high cholesterol and urinary problems in Latin America and Caribbean (Lans, 2006).

Mercier *et al.* (1999) used leaf and stem segments of *G. officinalis*, a medicinal pant, originated from aseptically grown seedlings to initiate cultures. Callus production was obtained on gelled Murashige and Skoog medium supplemented with 6-benzylaminopurine alone or combined with α-naphthalene acetic acid after 10 to 15 days of culture. The combination of 5.0 or 10.0 mg L^{-1} of 6-benzylaminopurine with 0.1 mg L^{-1} of α-naphthalene acetic acid were found to be best for shoot regeneration. While 10 mg L^{-1} of indole-3-butyric acid was considered optimal for the rooting of shoots. Vieire *et al.* (1995) described the production of fructose containing carbohydrates by leaf and node callus of *G. macrocephala* grown in different auxin to cytokinin ratios The amount of carbohydrates rose with increasing α-naphthalene acetic acid to 6-benzylaminopurine ratios, while in leaf callus it tend to decrease.

The main objective of this research was to evaluate the potential of different hormonal combinations on callus regeneration of *G. globosa* to optimize conditions for its propagation and to observe *in vitro* effect of different hormonal combinations on biochemical content in proliferated and regenerated calli.

MATERIALS AND METHODS

Culture media: Basal medium used in this study was MS medium (Murashige and Skoog, 1962) containing 30 g L^{-1} sucrose as a carbon source, 100 mg L^{-1} myoinositol, 0.4 mg L^{-1} thiamine HCl and 8 g L^{-1} agar. Five milliliter of MS medium was dispensed into each test tube and used in different combinations with BAP, NAA and GA_3. Ten combinations of each i.e., BAP+NAA and BAP+GA3 were used (Table 1).

Seed disinfection and *in vitro* culture: Seeds of *G. globosa* L. were surface sterilized by 0.1% solution of $HgCl_2$ and washed with distilled water 3-4 times. Seeds were inoculated on basal MS medium supplemented with

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Table 1: Effect of different concentrations of BAP+NAA and BAP+GA3 on callus characteristics and regeneration of *G. globosa* L

BAP+NAA (mg L^{-1})	Callus description			
	Proliferation	Color	Texture	Regeneration
0.5 + 1	+++	Greenish	Friable	NR
0.8 + 1	++	Greenish	Friable	NR
1 + 2	+++	Off white	Friable	NR
1 + 3	+++	Off white	Friable	NR
1.5 + 1	++	Off white	Friable	RI
2 + 0.2	++	Reddish	Friable	NR
2 + 0.5	++++	Brown	Friable	NR
4 + 0.2	++++	Reddish	Friable	NR
5 + 0.2	++++	Greenish	Friable	NR
6 + 0.5	++++	Greenish	Friable	NR
Control (MS)	++	Brown	Friable	NR
BBAP+GA_3 (mg L^{-1})				
0.5 + 1	++	Brown	Friable	RI
0.5 + 6	+++	Brown	Friable	RI
0.5 + 7	+++	Off white	Friable	RI+SI
1 + 0.2	+++	Brown	Friable	RI+SI
1 + 2	+++	Brown	Friable	RI
1 + 7	++	Greenish	Friable	RI
1 + 8	++	Greenish	Friable	RI
2 + 2	++++	Brown	Friable	NR
5 + 0.3	++++	Greenish	Friable	NR
5 + 0.5	++	Greenish	Friable	RI
Control (MS)	++	Brown	Friable	NR

NR: No Response; RI: Root Induction; SI: Shoot induction; ++++: Efficient callus proliferation; +++: Moderate callus proliferation; ++: Poor response

4 mg L^{-1} 2, 4-D and 10% coconut water (CW) for callus induction. Inoculated test tubes were incubated in Versatile Environmental Test Chamber (Sanyo MRL-350H) at 25±1°C under photoperiod of 16 h with light intensity of 50-55 μmol sec^{-2} m^{-1}. After 4 weeks off-white and friable calli were obtained that were inoculated on different media combinations (Table 1). Fifteen test tubes were inoculated at each media combination and incubated under conditions mentioned above for 5-6 weeks.

Biochemical analysis: Auxin, acid phosphatase activity and soluble protein content were estimated from proliferated and regenerated calli from different media combinations using the method of Mahadevan (1984), Iqbal and Rqfique (1987) and Lowry *et al.* (1951), respectively. Six replicates were selected from each media combination for the estimation of biochemical content.

Statistical analysis: For statistical analysis standard errors of the mean were calculated and means of different treatments were compared using Duncan's multiple range test ($p = 0.05$).

RESULTS AND DISCUSSION

Callus proliferation: After inoculation of callus on different media combinations with BAP and NAA poor response for regeneration was observed because most of the media combination showed various responses for callus proliferation. However, on some combinations root regeneration was observed. The most efficient callus proliferation was observed on 2 mg L^{-1} BAP +0.5 mg L^{-1} NAA, 4 mg L^{-1} BAP +0.2 mg L^{-1} NAA, 5 mg L^{-1} BAP +0.2 mg L^{-1} NAA, 6 mg L^{-1} BAP +0.5 mg L^{-1} NAA. In 0.5 mg L^{-1} BAP + 1 mg L^{-1} NAA, 0.8 mg L^{-1} BAP + 1 mg L^{-1} NAA, 1 mg L^{-1} BAP +2 mg L^{-1} NAA, 1 mg L^{-1} BAP +3 mg L^{-1} NAA, 1.5 mg L^{-1} BAP+1 mg L^{-1} NAA and 2 mg L^{-1} BAP+0.2 mg L^{-1} NAA moderate callus proliferation was observed. Different concentrations of BAP and NAA also influenced callus color/pigmentation because different responses for color such as greenish, off-white, brown and reddish were observed in different treatments and similar response for color was also observed with different combinations of BAP and GA3 with friable callus texture in all media combinations (Table 1). Different concentrations of BAP and NAA can influence callus development, color and callus friability (Jack *et al.*, 2005). Callus of *G. globosa* demonstrated the presence of pigments that might be suitable for further studies on the control of cell differentiation (Ruiz and Valadez, 1985).

Root induction: Callus of *G. globosa* L. was inoculated on different combinations of BAP +GA_3 to investigate the potential of organogenesis such as root and shoot regeneration. After 5-6 weeks of incubation period different responses for root and shoot regeneration and callus proliferation were observed on different concentrations of BAP and GA_3. In 0.5 mg L^{-1} BAP + 1 mg L^{-1} GA_3, 0.5 mg L^{-1} BAP+6 mg L^{-1} GA_3, 0.5 mg L^{-1} BAP+7 mg L^{-1} GA_3, 1 mg L^{-1} BAP+0.2 mg L^{-1} GA_3, 1 mg L^{-1} BAP+2 mg L^{-1} GA_3, 1 mg L^{-1} BAP + 7 mg L^{-1} GA_3, 1 mg L^{-1} BAP+8 mg L^{-1} GA_3 and 5 mg L^{-1} BAP +0.5 mg L^{-1} GA_3, root induction was observed. In 0.5 mg L^{-1} BAP + 7 mg L^{-1} GA_3 and 1 mg L^{-1} BAP + 0.2 mg L^{-1} GA_3. very low response for shoot induction was also observed.

Biochemical analysis: Different concentrations of BAP and NAA significantly enhanced endogenous IAA content of calli in most of the media treatments. Maximum increase in auxin was observed at 5 mg L^{-1} BAP+ 0.2 mg L^{-1} NAA that showed 146% increase in auxin content over control (Fig. 2a). Media combinations that showed high efficiency for callus proliferation also showed high auxin content. In BAP and GA_3 different media combinations also enhanced auxin content with maximum increase at 2 mg L^{-1} BAP+2 mg L^{-1} GA_3 where 130% increase in endogenous IAA content was observed

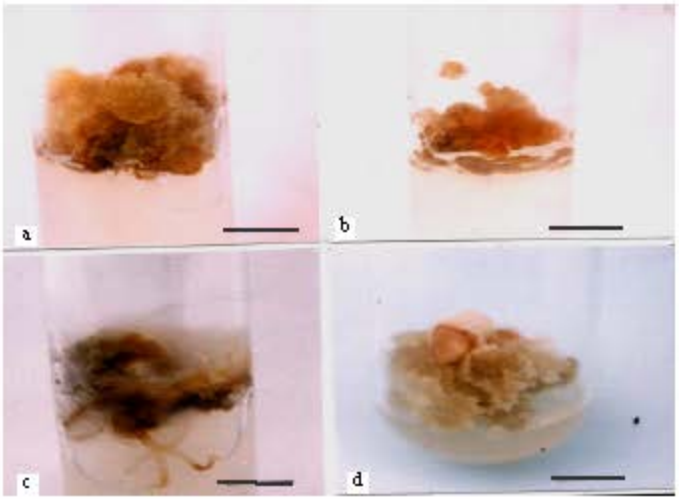

Fig. 1: Effect of different hormonal concentrations on callus characteristics and regeneration. (a) Efficient callus proliferation on 2 mg L^{-1} BAP+0.5 mg L^{-1} NAA with brown color and friable texture. (b) Red color callus on 2 mg L^{-1} BAP+0.2 mg L^{-1} NAA. (c) Root induction on 0.5 mg L^{-1} BAP+6 mg L^{-1} GA_3 and (d) Poor response for shoot induction on 0.5 mg L^{-1} BAP+7 mg L^{-1} GA_3. Bars are 1 cm

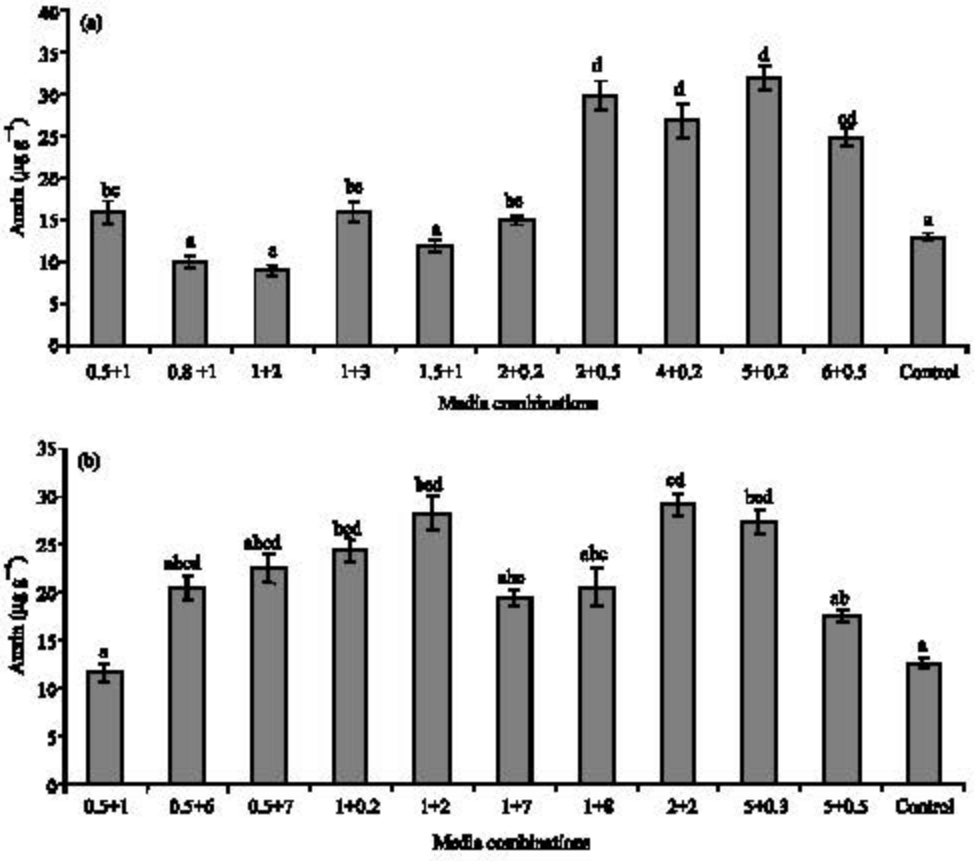

Fig. 2: Effect of different concentrations of hormones on endogenous IAA content of proliferated and regenerated calli. (a) Different combinations of BAP+NAA (mg L^{-1}). (b) Combinations of BAP+GA_3 (mg L^{-1}). Bars represent mean±SE of 6 replicates. Different letter(s) are statistically significant ($p = 0.05$)

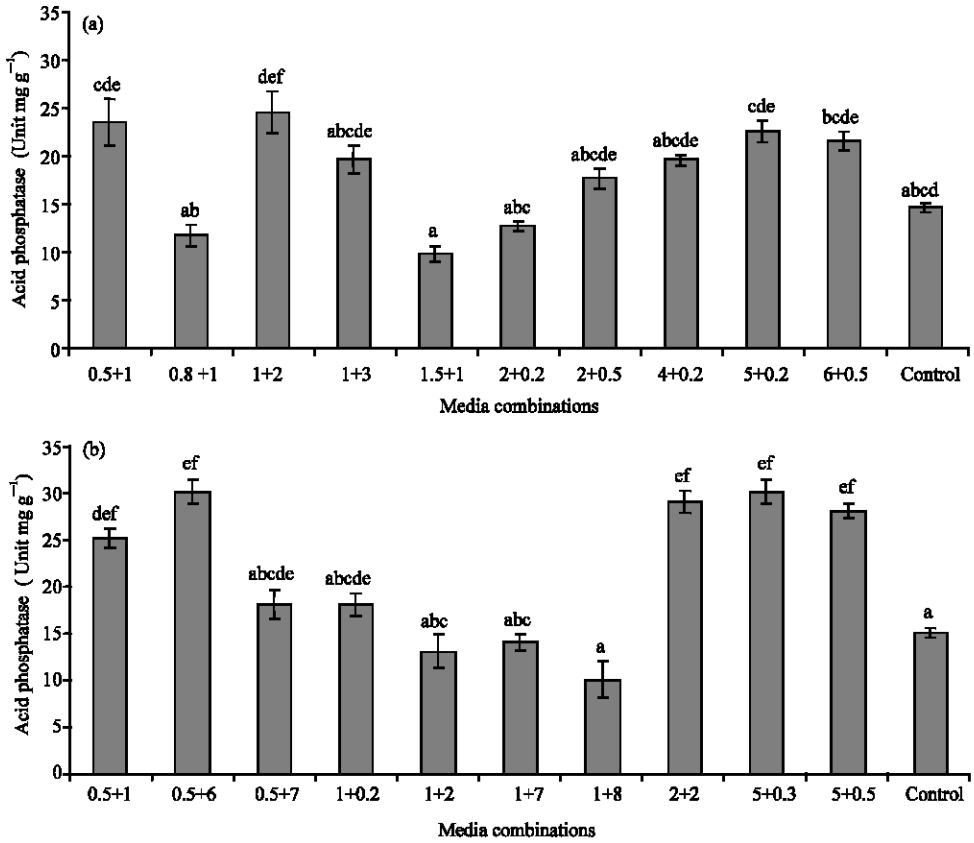

Fig. 3: Acid phosphatase activity in calli on different media combinations. (a) Different combinations of BAP+NAA (mg L^{-1}) and (b) Combinations of BAP+GA_3 (mg L^{-1}). Bars represent mean±SE of 6 replicates. Different letter(s) are statistically significant (p = 0.05)

over control (Fig. 2b). Higher concentrations of endogenous free IAA were found in embrogenic callus cultures growing on modified (Murashige and Skoog, 1962) medium (Jimernez and Bangerth, 2001). *In vitro* studies with *Quercus suber* have demonstrated that endogenous IAA concentrations significantly increased during early embryo development (Garcia-Martin *et al*., 2005).

In BAP and NAA most of the media combinations enhanced acid phosphatase activity over control. Maximum acid phosphatase activity was observed at 0.5 mg L^{-1} BAP+1 mg L^{-1} NAA and 1 mg L^{-1} BAP+2 mg L^{-1} NAA that showed 60 and 66% enzyme activity, respectively (Fig. 3a). In BAP and GA_3 maximum enzyme activity was observed at 0.5 mg L^{-1} BAP+6 mg L^{-1} GA_3 and 5 mg L^{-1} BAP+0.3 mg L^{-1} GA3 where 100% increase in acid phosphatase activity was observed (Fig. 3b).

In BAP and NAA most of the media concentrations have slightly increase the protein content over control. In 1 mg L^{-1} BAP+3 mg L^{-1} NAA and 5 mg L^{-1} BAP+0.2 mg L^{-1} NAA showed 19 and 17% increase in protein content, respectively (Fig. 4a) whereas in BAP and GA_3 maximum increase was observed at 1 mg L^{-1} BAP+0.2 mg L^{-1} GA_3 over control (Fig. 4b).

CONCLUSIONS

In the end it can be concluded that different concentrations of BAP, NAA and GA_3 in different media combinations have the potential for callus proliferation, root and shoot induction. Callus proliferation was observed on various concentrations of BAP (Fig. 4a and b) and NAA which can be further used for regenerations purpose whereas different concentrations of BAP and GA_3 have shown more potential for regeneration especially for root induction and in some combinations poor response for shoot induction was also observed which can be further investigated for *in vitro* propagation of *G. globosa* L. which is a valuable ornamental and medicinal plant.

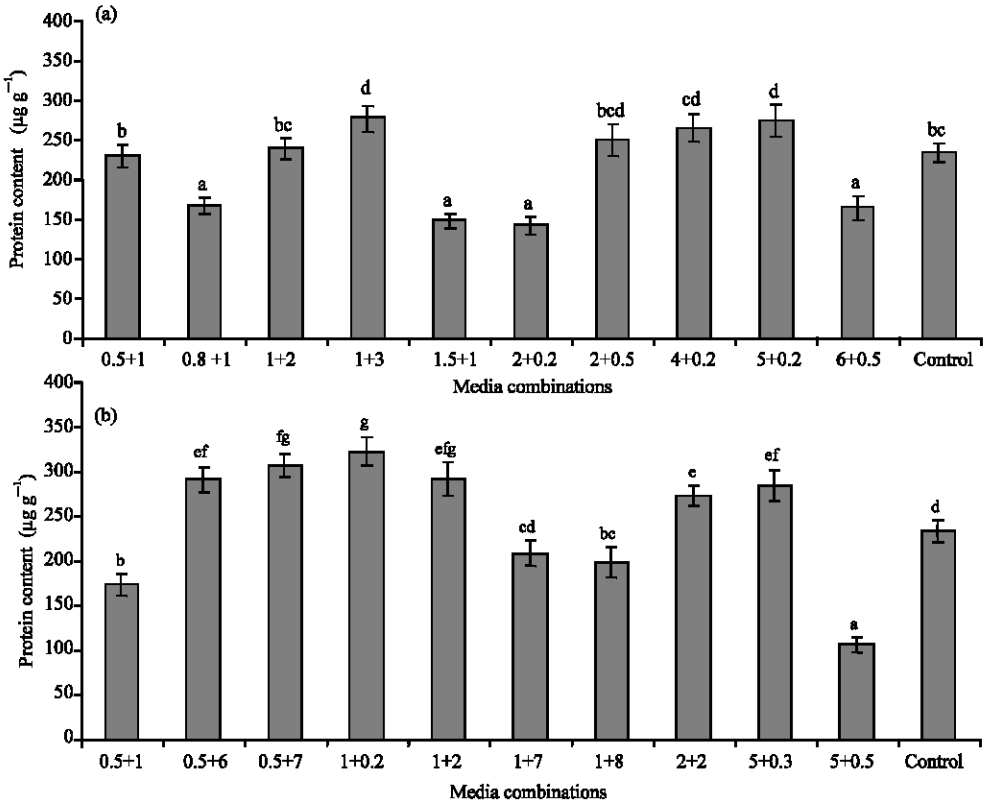

Fig. 4: Protein content of calli after inoculation on different media combinations. (a) Different combinations of BAP+NAA (mg L^{-1}) and (b) Combinations of BAP+ GA_3 (mg L^{-1}). Bars represent mean±SE of 6 replicates. Different letters are statistically significant (p = 0.05)

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