SCREENING OF DIFFERENT SPECIES (Table 4)

Three ornamentally important species of Dianthus viz. D. barbatus, D. caryophyllus and D. chinensis were screened to test their morphogenic competence. Viability of seeds was evaluated by culturing the seeds on half-strength MS medium. Seed viability of D. caryophyllus was 95% while that of D. barbatus was 70%. The seeds of D. chinensis did not germinate. So this species was discarded at this stage. Further D. caryophyllus and D. barbatus were tested for their morphogenic potential by culturing nodal explants from 21 days old aseptically germinated plants on MS medium containing BAP (0.5 mg l\(^{-1}\)). Percentage of responding explants and the total number of shoot-buds induced per explant were taken as criteria for assessing the morphogenic competence of Dianthus species. An average of 3 shoot buds was induced from nodal explants of D. caryophyllus (Fig. 2a) whereas only single shoot bud was induced in D. barbatus (Fig. 2b). Thus the regeneration response of D. caryophyllus was better than D. barbatus both in terms of percent response and total number of shoot buds induced per explant. Hence D. caryophyllus was selected for further studies.

1. EXPLANT CULTURE

1.1 Morphogenic response of seedling explants

The effect of different concentrations of cytokinins (BAP, Kn) and auxins (IAA, IBA, NAA, PAA) added to MS basal medium either alone or in combination was studied. Different explants viz. cotyledon, hypocotyl and cotyledonary node were excised from 10 day old aseptically germinated seedlings of D. caryophyllus.

1.1.1 Cotyledon

Effect of cytokinins (Table 5)

Effect of various concentrations of cytokinins viz. BAP or Kn (0.2-5 mg l\(^{-1}\)) on induction of shoot buds from cotyledon explants was studied. MS medium fortified with BAP or Kn failed to evoke any morphogenic response. The explant exhibited swelling after first week of culture and turned brown (Fig. 3a).
Effect of BAP in combination with auxins

Various concentrations of BAP (0.2-5 mg l\(^{-1}\)) in combination with IAA, IBA, NAA or PAA (0.2-5 mg l\(^{-1}\)) were tried for induction of shoot buds. MS medium fortified with BAP (0.2-5 mg l\(^{-1}\)) in combination with various auxins failed to evoke any morphogenic response. The explant exhibited swelling after first week of culture and turned brown.

1.1.2 Hypocotyl

Effect of cytokinins (Table 5)

Hypocotyl explants were cultured on MS medium supplemented with BAP or Kn (0.2-5 mg l\(^{-1}\)). Swelling of the explants was observed after two weeks of culture. MS medium supplemented with BAP (0.2 mg l\(^{-1}\)) at lower concentration did not induce any response. Light green watery callus was formed at the cut ends of the explant in the initial stages which later covered the whole explant (Fig. 3b). The amount of callus induced increased with the increasing concentration of BAP (0.5-3 mg l\(^{-1}\)). MS medium supplemented with higher levels of BAP (5 mg l\(^{-1}\)) resulted in formation of small amount of callus. Kn (0.2-5 mg l\(^{-1}\)) supplemented medium induced creamish callus only at the cut ends of the explant.

Effect of BAP in combination with auxins (Table 6)

Hypocotyl segments cultured on different combinations of BAP (0.2-5 mg l\(^{-1}\)) with IAA, IBA, NAA or PAA (0.2-5 mg l\(^{-1}\)) gave callusing response. Medium supplemented with lower levels of BAP (0.2-0.5 mg l\(^{-1}\)) with IAA, IBA or NAA ((0.2-5 mg l\(^{-1}\)) induced small amount of callus. Light green watery callus was induced at the basal cut ends of hypocotyl explants on higher level of BAP (1-5 mg l\(^{-1}\)) in combination with IAA, IBA or NAA (0.2-5 mg l\(^{-1}\)). Combination of BAP and PAA could not evoke any morphogenic response from the explants.

1.1.3 Cotyledonary node

Effect of cytokinins (Table 5)
Effect of cytokinins viz. BAP or Kn (0.2-5 mg l\(^{-1}\)) was studied on induction of shoot buds from cotyledonary node explants. An average of 2 shoot buds per explant along with callus was induced on MS medium with lower concentrations of BAP (0.2-1 mg l\(^{-1}\)) (Fig. 3d). Concomitant decrease in number of shoot buds was observed with increase in the concentration of BAP (3-5 mg l\(^{-1}\)) in the medium. Kn (0.5-5 mg l\(^{-1}\)) supplemented medium induced green and compact callus from the cotyledonary node explants that showed no morphogenic response on subculturing (Fig. 3e). No shoots were regenerated from cotyledonary node explants on Kn supplemented medium.

**Effect of BAP in combination with auxins (Table 6)**

Cotyledonary node explants were cultured on different concentrations of BAP (0.2-5 mg l\(^{-1}\)) in combination with IAA, IBA, NAA or PAA (0.2-5 mg l\(^{-1}\)) to observe the morphogenic potential. MS medium supplemented with lower concentrations of BAP (0.2-0.5 mg l\(^{-1}\)) in combination with lower concentration of IAA, IBA or NAA (0.2-0.5 mg l\(^{-1}\)) induced an average of 2 shoot buds per explant (Fig. 3f, g). Further increasing the level of BAP up to 1 mg l\(^{-1}\) and IAA, IBA or NAA (0.2-1 mg l\(^{-1}\)) in factorial combinations, induced single shoot bud. BAP (0.2-5 mg l\(^{-1}\)) in combination with PAA (0.2-5 mg l\(^{-1}\)) did not evoke any morphogenic response. Higher levels of BAP (3-5 mg l\(^{-1}\)) and auxins (0.2-5 mg l\(^{-1}\)) did not promote shoot regeneration.

### 1.2 Morphogenic response of mature plant explants

The effect of different concentrations of cytokinin (BAP, Kn) and auxins (IAA, IBA, NAA, PAA) added to MS basal medium either alone or in combination was studied. Different explants viz. shoot tip, leaf, internode and node were excised from 21 day old aseptically raised mature plants of *D. caryophyllus*.

#### 1.2.1 Shoot tip

**Effect of cytokinins (Table 7)**

Shoot tips excised from *in vitro* raised plants were cultured on medium supplemented with BAP or Kn (0.2-5 mg l\(^{-1}\)). On lower levels of BAP (0.2-0.5 mg l\(^{-1}\)) multiple shoots were formed by proliferation of existing meristem. On raising the levels of BAP (1-5 mg l\(^{-1}\)), elongation of single shoot was observed. Elongation of shoot tip was
observed on Kn (0.2-5 mg l⁻¹) supplemented medium. Thus BAP was found to be superior than Kn in evoking morphogenic response from shoot tip explants.

**Effect of BAP in combination with auxins (Table 9,10)**

Lower levels of BAP (0.2 mg l⁻¹) in combination with IAA (0.2-1 mg l⁻¹) formed 1-2 shoot buds after 2 weeks of incubation. Organogenic response was observed on higher level of BAP (0.5 mg l⁻¹) with IAA (0.5 mg l⁻¹) where 2-3 shoot buds were formed. On medium supplemented with BAP (0.2 mg l⁻¹) and IBA (0.2-1 mg l⁻¹), slight elongation along with 1-2 small axillary shoot buds were induced. No profound effect of BAP (0.5-1 mg l⁻¹) in combination with IBA (0.5-1 mg l⁻¹) was observed. Also PAA in combination with BAP did not give a good response. Elongation of single shoot along with callusing was obtained on lower levels of BAP (0.2 mg l⁻¹) in combination with PAA (0.2-1 mg l⁻¹). Higher levels of BAP (0.5-1 mg l⁻¹) promoted formation of 1-2 shoots accompanied with slight callusing. Medium supplemented with BAP in combination with NAA gave better response than all other combinations. Best response was observed on medium supplemented with BAP (0.5 mg l⁻¹) in combination with NAA (0.5 mg l⁻¹) on which 3-4 shoot buds were formed.

**1.2.2 Leaf**

**Effect of cytokinins (Table 7)**

Leaves excised from 1st to 3rd nodes from the shoot apex were cultured on medium supplemented with BAP or Kn (0.2-5 mg l⁻¹). Swelling of the explant was observed after first week of incubation on BAP (0.2-1 mg l⁻¹) supplemented medium. Callus formation was observed on all the concentrations of BAP or Kn. The callus was compact, green and nodular (Fig. 3c). The callus was further subcultured on various combinations of PGRs but it failed to exhibit any morphogenic response.

**Effect of BAP in combination with auxins (Table 9,10)**

Various levels of BAP (0.2-1 mg l⁻¹) in combination with different concentrations of auxins viz. IAA, IBA, NAA or PAA (0.2-1 mg l⁻¹) evoked callusing response from leaf explants. Swelling of the explant was observed in the first week of culture. Green, compact and non-morphogenic callus was observed at the basal cut ends of the leaves in the second week of incubation. Medium supplemented with BAP with higher levels of
auxins (1 mg l⁻¹) formed maximum amount of callus. Callus was initiated at the cut ends of the explants after 2⁰ week of incubation. BAP with lower levels of auxins (0.2-0.5 mg l⁻¹) resulted in formation of small amount of callus only at the cut ends of the explant.

1.2.3 Internode (Table 7)

Internodal segments of 0.5- 0.8 cm in length were cultured on MS medium supplemented with BAP or Kn (0.2-5 mg l⁻¹) alone and on BAP (0.2-5 mg l⁻¹) in combination with IAA, IBA, NAA or PAA (0.2-5 mg l⁻¹). No morphogenic response was seen on any of the hormonal combinations tried.

1.2.4 Node

Effect of cytokinins (Table 8)

Nodal explants (1ˢᵗ to 3ʳᵈ nodes from shoot apex) were excised from in vitro raised plants and cultured on MS medium supplemented with BAP or Kn (0.2-5 mg l⁻¹). Medium supplemented with lower levels of BAP (0.2 mg l⁻¹) resulted in elongation of the axillary buds. On raising the level of BAP (0.5 mg l⁻¹), multiple shoots were formed from the explant (Fig. 4a). Higher levels of BAP (1-5 mg l⁻¹) did not support regeneration. Formation of 1-2 shoot buds along with callusing was obtained on medium supplemented with Kn (0.2-1 mg l⁻¹). The callus was green and nodular which did not regenerate on further subculture (Fig. 4b). Nodal explants did not exhibit any response when subcultured on higher levels of Kn (3-5 mg l⁻¹). Thus BAP supplemented medium was found to be better than Kn in evoking morphogenic response from the nodal explants.

Effect of BAP in combination with auxins (Table 11)

Nodal explants were cultured on various concentration of BAP (0.2-5 mg l⁻¹) in combination with auxins viz. IAA, IBA, NAA or PAA (0.2-5 mg l⁻¹) for testing their morphogenic potential. Swelling of explant was observed after first week of incubation. Lower levels of BAP (0.2-0.5 mg l⁻¹) in combination with IAA or IBA (0.2-5 mg l⁻¹) resulted in formation of 1-2 shoot buds (Fig. 4c, d). Higher levels of BAP (5 mg l⁻¹) together with IAA or IBA failed to exhibit any morphogenic response from the nodal explant. Combination of BAP and PAA were not supportive for organogenesis. Shoot buds formed on medium supplemented with BAP (0.2-0.5 mg l⁻¹) with PAA (0.2-1 mg l⁻¹)
1) resulted in the formation of 1-2 shoot buds. The shoots formed were thin and slender (Fig. 4f). Medium supplemented with BAP and NAA was found to be optimum for regeneration as multiple shoots were formed in all the factorial combinations. Best response was observed on medium supplemented with BAP (0.5 mg l\(^{-1}\)) and NAA (0.5 mg l\(^{-1}\)) on which 4-5 shoot buds were formed. The shoots obtained were well elongated with their length ranging from 3-4 cm (Fig. 4e).

2. PROLIFERATION OF SHOOTS (Table 12)

The shoot buds induced from various explants were sectored into clumps of 1-3 shoots and subcultured on medium supplemented with BAP (0.2-1 mg l\(^{-1}\)) and auxins viz. IAA, IBA, NAA or PAA (0.2-1 mg l\(^{-1}\)). The shoot buds obtained from various explants viz. cotyledonary node and shoot tip did not show any proliferation when subcultured on different PGRs supplemented medium. Multiple shoots obtained from nodal explants on medium supplemented with BAP and IAA or IBA when subcultured on medium with same level of PGRs resulted in sprouting of new buds (Fig. 4g, h). Transfer of shoots to higher concentrations of BAP (1 mg l\(^{-1}\)) with different auxins did not promote the proliferation of primary shoots. Subculture of shoot buds to medium supplemented with BAP and PAA did not support proliferation of shoots (Fig. 4j). Best proliferation response was obtained when shoot buds induced on medium supplemented with BAP (0.5 mg l\(^{-1}\)) and NAA (0.5 mg l\(^{-1}\)) were subcultured on same levels of PGRs in the medium (Fig. 4i). Abnormal hyperhydric shoots were also obtained along with normal, healthy shoots on all the combinations of proliferation medium. These shoots were glassy, water-soaked, light green in colour and abnormal. They did not survive well and were thus discarded. The number of hyperhydric shoots is not included in the tabular results. Organogenic cultures could be maintained for only 3-4 subcultures, but as the number of subculture increased, the problem of hyperhydricity also increased.

3. EFFECT OF GELLING AGENTS

The type and concentration of gelling agent used to solidify the medium also had profound effect on shoot morphogenesis. Various concentrations of agar (0.9-1.4%), CleriGel (0.1-0.5%) and isabgol (1-4%) were tried (Fig. 5a-d). Regeneration medium solidified with isabgol was not effective in reducing the number of hyperhydric shoots.
There was a reduction in the number of shoots regenerated thus isabgol suppressed the morphogenic response as well. Regeneration medium solidified with CleriGel was successful in reducing the number of hyperhydric shoots. Best response was obtained when percentage of agar was increased to 1.2% in the regeneration medium. At this level, the number of hyperhydric shoots was reduced to a large extent. However, agar (1.2%) failed to reduce hyperhydricity with 100% efficiency.

4. EFFECT OF INORGANIC AND ORGANIC NUTRIENTS AND OTHER MEDIA SUPPLEMENTS

Effect of various inorganic and organic nutrients present in the MS basal medium was investigated. The concentration of each component of the MS medium was modified one at a time to observe the effect of individual nutrient on induction and proliferation of shoot buds. Some other media additives such as sugars, heavy metals and growth adjuvants were also added to the basal medium with similar objective.

4.1 Effect of inorganic nutrients

Ammonium Nitrate (NH$_4$NO$_3$)

Nodal segments were cultured on MS medium supplemented with BAP (0.5 mg l$^{-1}$) + NAA (0.5 mg l$^{-1}$) and different levels of NH$_4$NO$_3$ (0, 5.15, 10.3, 20.61*, 41.2 mM) (Table 13, Fig. 6a-d). NH$_4$NO$_3$ supplies nitrogen both in oxidized and reduced form. Shoot buds induced in the absence of NH$_4$NO$_3$ were weak and abnormal which could not form healthy shoots on subculture on proliferation medium. MS level of NH$_4$NO$_3$ (20.61 mM) was found to be optimal for induction and proliferation of shoots. Reducing the level of NH$_4$NO$_3$ to one-fourth of the normal MS level i.e. 5.15 mM resulted in the formation of 9-10 healthy shoots per explant. The number was comparable to the control cultures but no hyperhydric shoot formation accompanied the normal shoot morphogenesis therefore hyperhydricity was controlled with 100% efficiency. Thus, 5.15 mM of NH$_4$NO$_3$ was considered optimum for Dianthus cultures.

Potassium Nitrate (KNO$_3$)
Nodal segments were cultured on MS medium supplemented with BAP (0.5 mg l\(^{-1}\)) + NAA (0.5 mg l\(^{-1}\)) and different levels of KNO\(_3\) (0, 9.4, 18.8*, 37.6, 56.4 mM) (Table 14, Fig. 6e-h). KNO\(_3\) serves as an alternative source of oxidized nitrogen and a major source of potassium ions. Elimination of KNO\(_3\) from the induction medium led to the formation of weak and light green shoot buds, which did not proliferate on subculture. Reducing the concentration of KNO\(_3\) to half of the normal MS level (9.4 mM), resulted in the formation of 2-3 abnormal shoots which did not proliferate normally on subculture. The MS level of KNO\(_3\) formed normal, healthy shoot buds and proved to be optimal for proliferation. Increase in the level of KNO\(_3\) upto 56.4 mM resulted in reduced shoot bud formation and callusing.

**Boric Acid (H\(_3\)BO\(_3\))**

Nodal segments were cultured on MS medium supplemented with BAP (0.5 mg l\(^{-1}\)) + NAA (0.5 mg l\(^{-1}\)) and different levels of H\(_3\)BO\(_3\) (0, 0.1*, 0.2, 0.3, 0.4, 0.5 mM) (Table 15, Fig. 7a-d). H\(_3\)BO\(_3\) in the culture medium supplies boron to the culture system. Deficiency of boron in culture medium did not prove to be favourable for shoot bud induction. Raising the levels of H\(_3\)BO\(_3\) to 2-4 times the MS levels at 0.2 mM and 0.4 mM H\(_3\)BO\(_3\) respectively, the number of shoots reduced to half than that of the control. These shoot buds failed to proliferate into normal shoots on subculture. Further elevated levels of H\(_3\)BO\(_3\) up to 0.5 mM did not promote shoot induction at all.

**Potassium Dihydrogen Phosphate (KH\(_2\)PO\(_4\))**

Nodal segments were cultured on MS medium supplemented with BAP (0.5 mg l\(^{-1}\)) + NAA (0.5 mg l\(^{-1}\)) and different levels of KH\(_2\)PO\(_4\) (0, 1.25*, 2.5, 3.75, 5.0, 6.25, 12.5 mM) (Table 16, Fig. 7e-h). KH\(_2\)PO\(_4\) is the sole source of phosphate in the medium. This compound supplies K\(^+\) in very low concentrations while the major source of K\(^+\) is KNO\(_3\). Absence of KH\(_2\)PO\(_4\) from the induction medium failed to induce any shoot buds from the explants. Shoot buds obtained on medium with 2.5 mM of KH\(_2\)PO\(_4\) (double the MS level) were abnormal and on subculturing for proliferation to MS levels of KH\(_2\)PO\(_4\) did not form normal shoots. Elevated concentrations of KH\(_2\)PO\(_4\) did not prove favourable for induction as well as proliferation of shoots. Maximum number of 9-10 shoots was formed on MS levels of KH\(_2\)PO\(_4\).
Potassium Iodide (KI)

Nodal segments were cultured on MS medium supplemented with BAP (0.5 mg l$^{-1}$) + NAA (0.5 mg l$^{-1}$) and different levels of KI (0, 5*, 10, 15, 20, 25, 35 µM) (Table 17, Fig. 8a-d). KI is the source of iodine in the medium. It does not contribute much to the K$^+$ concentration. Presence of KI is essential for the induction of normal shoots from the explant as no shoot buds were induced on medium devoid of KI. MS levels of KI were found to be optimal for induction and proliferation of shoots in Dianthus cultures. Increase in KI concentration resulted in formation of hyperhydric shoots.

Cobalt Chloride (CoCl$_2$)

Nodal segments were cultured on MS medium supplemented with BAP (0.5 mg l$^{-1}$) + NAA (0.5 mg l$^{-1}$) and different levels of CoCl$_2$ (0, 0.11*, 0.22, 0.55, 1.1, 2.2, 5.5 µM) (Table 18, Fig. 8e-h). CoCl$_2$ is an important and sole source of cobalt in the medium. An average of 2 shoots was formed on induction medium devoid of CoCl$_2$. CoCl$_2$ exerted a profound influence on differentiation of shoot buds. There was a constant increase in the number of shoot buds till the level of CoCl$_2$ was optimized at 0.55 µM. An average of 18 shoots per explant was obtained when shoot buds induced on induction medium containing 0.55µM CoCl$_2$ were subcultured on similar proliferation medium. Two-fold increase in the number of shoots as compared to the control was witnessed.

Sodium Molybdate (Na$_2$MoO$_4$)

Nodal segments were cultured on MS medium supplemented with BAP (0.5 mg l$^{-1}$) + NAA (0.5 mg l$^{-1}$) and different levels of Na$_2$MoO$_4$ (0, 1.03*, 5.15, 10.3, 20.6, 30.9 µM) (Table 19, Fig. 9a-d). Na$_2$MoO$_4$ being an important and sole source of molybdenum plays a crucial role in in vitro morphogenesis. The shoot buds developed on medium devoid of Na$_2$MoO$_4$ when subcultured on similar medium, did not proliferate and when subcultured on medium with MS level of Na$_2$MoO$_4$ (1.03 mM) formed few shoots. Thus Na$_2$MoO$_4$ level in the induction medium is critical for proliferation of shoot buds. Maximum differentiation of shoot buds was observed at MS levels of Na$_2$MoO$_4$. Higher levels of this nutrient were not found to be beneficial.

Calcium Chloride (CaCl$_2$)
Nodal segments were cultured on MS medium supplemented with BAP (0.5 mg l\(^{-1}\)) + NAA (0.5 mg l\(^{-1}\)) and different levels of CaCl\(_2\) (0, 2.99*, 5.98, 8.97, 11.96, 14.95, 29.9 mM) (Table 20, Fig. 9e-h). CaCl\(_2\) is the sole source of calcium in the culture media. CaCl\(_2\) in the induction medium was found to be an essential component as shoot buds did not form in the absence of CaCl\(_2\). Shoot buds induced on 5.98 mM CaCl\(_2\) (double the MS level) in the induction medium and subcultured on similar level of CaCl\(_2\) (5.98 mM) in the proliferation medium yielded maximum number of shoots. Thus, optimum concentration of CaCl\(_2\) in induction as well as proliferation medium was found to be 5.98 mM for *Dianthus* cultures.

**Magnesium Sulphate (MgSO\(_4\))**

Nodal segments were cultured on MS medium supplemented with BAP (0.5 mg l\(^{-1}\)) + NAA (0.5 mg l\(^{-1}\)) and different levels of MgSO\(_4\) (0, 1.5*, 3.0, 4.5, 7.5, 15.0 mM) (Table 21, Fig. 10a-d). MgSO\(_4\) is the sole source of magnesium. Absence of MgSO\(_4\) from the induction medium failed to induce normal shoot buds. Elevated concentrations of MgSO\(_4\) proved beneficial than the MS concentrations. The optimum concentration of MgSO\(_4\) for shoot bud induction was found to be 3 mM i.e. double the MS levels. The shoots obtained were green, less hyperhydric, sturdy and on subculture at similar level of MgSO\(_4\), resulted in healthy and increased number of shoots. However, further increase of MgSO\(_4\) levels up to 7.5 mM, induced less number of shoots which on proliferation formed hyperhydric shoots accompanied with callusing.

**Zinc Sulphate (ZnSO\(_4\))**

Nodal segments were cultured on MS medium supplemented with BAP (0.5 mg l\(^{-1}\)) + NAA (0.5 mg l\(^{-1}\)) and different levels of ZnSO\(_4\) (0, 29.91*, 59.82, 89.73, 119.64, 149.55, 299.1 µM) (Table 22, Fig. 10e-h). ZnSO\(_4\) is the source of micronutrient zinc in the culture medium. Absence of zinc in the medium resulted in the formation of 1-2 shoots which did not proliferate well on subculture. The MS level of ZnSO\(_4\) (29.91 µM) was considered optimum for induction of shoot buds with an average of 5 shoot buds induced per explant. The ZnSO\(_4\) concentration in the basal medium proved to be optimum for developing maximum number of shoots per explant. Dramatic differences were observed when shoot buds induced on a particular concentration of ZnSO\(_4\) were
subcultured on medium with similar ZnSO₄ concentration or MS levels of ZnSO₄. Shoot buds induced on 89.73, 119.64 or 149.55 µM of ZnSO₄ when subcultured on medium with normal level of ZnSO₄, failed to proliferate and in contrast, showed abnormalities like vitrified leaves and stunted growth. When subcultured to a medium with modified ZnSO₄, the shoot buds proliferated to produce normal shoots but less in number.

**Copper Sulphate (CuSO₄)**

Nodal segments were cultured on MS medium supplemented with BAP (0.5 mg l⁻¹) + NAA (0.5 mg l⁻¹) and different levels of CuSO₄ (0, 0.1*, 1, 2, 3, 5, 10µM) (Table 23, Fig. 11a-d). CuSO₄ supplies the micronutrient copper in the culture medium. Induction of shoots was observed on the medium devoid of CuSO₄, but they were less in number, light green and unhealthy. Higher levels of copper in the induction as well as proliferation medium resulted in formation of increased number of shoots. There was a concomitant increase in the number of shoot buds to up to 10 shoots per explant on increasing the concentration up to 5 µM CuSO₄ (50 times the normal MS level). On further subculture to similar concentration of CuSO₄ (5 µM), the number increased to 24 shoots per explant which is approximately 2.5 fold more than that obtained in control. Higher CuSO₄