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*Full Length Research Paper*

# Effectiveness of tissue culture media components on the growth and development of cauliflower (*Brassica oleracea* var. Botrytis*)* seedling explants *in vitro*

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**A key factor in the application of *in vitro* techniques to cauliflower improvement is the development of efficient protocols for regeneration of plants from tissue for use in breeding programs for the selection of the desirable genotypes under biotic and abiotic stress. Experiments were conducted to study the effect of different media components (agar or agar + sucrose or agar + Murashige and Skoog (MS) salts or agar + sucrose + MS) on callus induction and regeneration from five explants types (cotyledon, hypocotyls, shoot apex, primary root and root tip) and also the effects of auxin and cytokinin were carried out using one $F_1$ hybrid cauliflower cv. Medallion. The results show that cotyledons, mid roots and root apices grown on agar + MS + sugar were the most developed compared with explants on the other media. This medium was the most productive in terms of lateral root number and root length. The presence of 2,4-dichlorophenoxyacetic acid increased callus production compared to 6-benzylamino purine. Within the selected explants, a significant difference was indicated between different explants under different treatments. Liquid culture was more successful at producing viable plantlets than solid culture.**

**Key words:** Cauliflower, explants, *in vitro*, growth regulator, growth characters, solid and liquid culture.

## INTRODUCTION

Cauliflower is valued as a vegetable as it contains high amounts of vitamins C, K and A, also folic acid, fiber and flavonoids which gives cauliflower its antioxidant and anti-inflammatory properties and is important to human and animal. Eating adequate amount of cauliflower can lower the risk of cancer, particularly bowel, breast and other female cancers. It is grown through the world production in 2011 of over 18 million tones (http://faostat.fao.org).

As world population is expected to reach 8.5 billion by 2025 (Chrispeels and Sadava, 2003), there is a dire need for increased food production. Abiotic stresses such as drought, salinity and high and low temperatures limit crop productivity, and play a major role in determining the distribution of plant species across different type of environments (Metwali, 2012). Improvement of cauliflower in both direction –quantity and quality- are occurs by either conventional methods or biotechnological methods under normal and stress conditions (Bhalla and Smith, 1998; Newell and Burke, 2000; Chrispeels and Sadava, 2002; Lin et al., 2010).

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**Abbreviations: 2,4-D,** 2,4-Dichlorophenoxyacetic acid; **BAP,** 6-Benzylamino purine; **MS,** Murashige and Skoog.

In the 1920's, plant tissue culture was initially used as a technique for germination orchid seed. The science of plant tissue culture has been developed further and plant tissue culture is now used to propagate many ornamental and crop species (George and Sherrington, 1984; Gregory, 1999; Kiarash et al., 2012). The opportunities for rapid crop improvement have increased dramatically since 1970's with development of plant tissue culture. The essential in gradients of plant tissue culture media can be grouped into six classes, macronutrients, micronutrients, vitamin, amino acid, organic acid and sugar addition to plant growth regulator. Tissue culture method of micro propagation of *Brassica oleracea* can provide a greater way for rapid propagation (Qin et al., 2006).

*In vitro* regeneration of cauliflower is possible from different explants (Kirti et al., 2001; Ying et al., 2006). To perform efficient calli production for regeneration and gene cloning of cauliflower is necessary to optimize calli culture condition, which involve explants types (Kerlley et al., 2012), media component (Narpal et al., 2006; Ovesna et al., 2006), plant growth regulator (Hoque, 2010) and liquid medium (Modarres and Jami, 2003). To initiate growth of the cauliflower explants, it is important to provide basic nutrients within the media, this usually consists of a mixture of salts which provide the essential macro and micro elements as well as carbon source, usually sucrose (Dita et al., 2011). The most widely used of the formulations available is Murashige and Skoog media (Akin-Idowu et al., 2009). Growth regulators are used to support a basic level of growth but are equally important to direct the developmental response of the Propagule (Kerlley et al., 2012).

The tissue culture method is a novel approach to the improvement of genotypes under drought, salinity and extreme temperature and can exploit maximum genetic gain achieved in breeding programs (Abdel-Raheem et al., 2007; Akin-Idowu et al., 2009; Julia and Claudia, 2012). Taking this information into consideration, the present study was undertaken to optimize tissue culture protocol of cauliflower for its use in cauliflower breeding programs for the evaluation and selection of desirable genotypes from cauliflower under abiotic and biotic stress

## MATERIALS AND METHODS

### Role of agar, sugar and nutrients on the growth and development of cauliflower seedling explants

$F_1$ hybrid cauliflower Medallion was used in this study. Under aseptic *in-vitro* culture conditions, seedlings were dissected into five different types of explants and each explants was culture separately on four different types of media: (a) agar (7 g/L), (b) agar (7 g/L) + MS nutrient (4.4 g/L), (c) agar (7 g/L)+ sugar (30 g/L) and(d) agar (7 g/L) + MS nutrient (4.4 g/L) + sugar (30 g/L). All Petri dishes were placed under a light bank for propagation. Number of lateral roots produced on mid-root, hypocotyls and length of the root tip section were recorded every ten days (10, 20, 30 and 40 days).

### Role of auxin and cytokinin on the growth and development of cauliflower seedling hypocotyls explants

Five hypocotyls explants were placed in three replicate plates of each of the following sterile treatments: (a) agar (7 g/L) + MS (4.4 g/L) + sugar (30 g/L), (b) agar (7 g/L) + MS ( 4.4 g/L) + sugar (30 g/L)+ 2,4-D (0.002 g/L), (d) agar (7 g/L) + MS (4.4 g/L) + sugar (30 g/L) +BAP (0.003 g/L) and (d) agar (7 g/L) + MS (4.4 g/L) + sugar (30 g/L) + 2,4-D(0.002 g/L) + BAP (0.003 g/L). The amount of callus present was assessed visually after four weeks on a scale of 0 to 4 where 0 = no callus, and 4 = large callus.

### Comparison of solid and liquid culture for the production of shoot from cauliflower curd

A cauliflower curd was taken and an aseptic procedure was applied. The prepared meristem pieces awere then placed in equal amounts in five pots containing S23 [(gar (7 g/L) + MS (4.4 g/L) + sucrose (30 g/L) + 2,4-D (0.002 g/L) + adenine (0.080 g/L) + thiamine (0.0004 g/L) + sodium phosphate (0.170 g/L)] solid medium and five pots containing S23 liquid medium with three replicates for each media. The medium pots were every ten days periodically observed with any obvious differences recorded.

### Statistical analysis

Analysis of variance (ANOVA) was employed, means compared using the least significant difference (LSD) and correlation was calculated according to Gomez and Gomez (1984) and Mode and Robinson (1959), respectively.

## RESULTS AND DISCUSSION

### Influence of agar, sugar and nutrients

From the experiment carried out, it can be seen that the cotyledons, mid roots and root apex's grown in the A + MS + sugar were the most developed compared with plant tissues in the other growth medium. This was followed by the agar and sugar in all cases expect for cotyledon explants, (Table 1 and Figure 1), where the next best media for growth and proliferation was the agar with MS nutrients. The greater degree of growth and development of the plant tissues in the A + MS + sugar growth media is likely to be due to the greater amount of nutrients, mineral and vitamins A available to the plant tissues. The mineral are important for three main types of function; first, some mineral elements are essential components of molecule vital to plant life; secondly, mineral elements may be directly required in plant cell metabolism; thirdly, some minerals, in the form of ions, preserve various equilibrium in the plant cell (Forbes and

**Table 1.** Mean performance of different component media on the growth different explants after 0, 10, 20 and 30 days

| Genotype | Date (day) | Type of media | Trait | | | | |
|---|---|---|---|---|---|---|---|
| | | | Cotyledon length (cm) | Hypocotyl length (cm) | Shootapex length (cm) | Primaryroot length (cm) | Roottip length (cm) |
| Medallion | 0 | Agar (7 g/L) | 0.4 | 1.3 | 0.5 | 1.2 | 0.4 |
| | 10 | | 0.6 | 1.4 | 0.5 | 1.3 | 0.4 |
| | 20 | | 0.6 | 1.5 | 0.6 | 1.5 | 0.5 |
| | 30 | | 0.7 | 1.4 | 0.7 | 1.6 | 0.6 |
| Medallion | 0 | Agar (7 g/L) + MS (4.4g/L) | 0.4 | 1.3 | 0.5 | 1.2 | 0.4 |
| | 10 | | 0.5 | 1.4 | 0.5 | 1.3 | 0.5 |
| | 20 | | 0.5 | 1.5 | 0.6 | 1.3 | 0.5 |
| | 30 | | 0.5 | 1.6 | 0.6 | 1.4 | 1.6 |
| Medallion | 0 | Agar (7 g/L) + sucrose (30 g/L) | 0.4 | 1.3 | 0.5 | 1.2 | 0.4 |
| | 10 | | 0.7 | 1.5 | 0.6 | 1.7 | 0.7 |
| | 20 | | 0.1 | 1.7 | 0.7 | 1.8 | 0.9 |
| | 30 | | 1.5 | 1.8 | 0.8 | 2.00 | 1.3 |
| Medallion | 0 | Agar (7 g/L) + MS (4.4 g/L ) + sucrose (30 g/L) | 0.4 | 1.3 | 0.5 | 1.2 | 0.4 |
| | 10 | | 0.8 | 1.6 | 0.9 | 1.2 | 0.6 |
| | 20 | | 1.6 | 1.9 | 1.7 | 1.7 | 1.1 |
| | 30 | | 2.2 | 1.9 | 2.7 | 2.2 | 1.5 |
| LSD at 0.05 | | | 0.051 | 0.140 | 0.083 | 0.0720 | 0.090 |

Watson, 1996*).* There were no lateral roots presenting any of the cauliflower cotyledons grow in A +sugar after 30 days (Figure 2a), while there was an average of 0.6 roots per cotyledon in the agar + MS, 3 roots per cotyledon in the A + MS + sugar. After 30 day, there was an average of 0.2 roots per cotyledon in the agar media.

The A + MS + sugar growth media had the greatest number of lateral roots per mid-root segment after 30 days with an average of 3.5 roots per segment (Figure 2b). The mid – root segment grown in A + sugar produced the second highest average of 2.0 root per segment. The mid-root segment grown in the agar did not produce any lateral roots. Although the A + sugar had significant effect on the cotyledons and hypocotyls, it still proved to be the second most productive growth media in terms of the number of lateral roots per mid-root segment and the root apex length. This would indicate that the sugar provide more of essential and minerals to the explants than the MS nutrient. However, the combination of MS nutrients and agar proved to have the greatest level of response resulting in new cotyledons developing on the hypocotyls, shoot apex and cotyledons. Within the selected explants, there was a high significant difference and positive high significant was indicated between different explants under different treatments (Tables 2 and 3).

The shoot apex grew the most substantially out of all the explants thus showing the highest degree of tot potency, this could have been due to the fact it is a shoot meristem and contains apical cells, there is a greater number of growing and dividing cells in the shoot apex than root apex and so the absolute growth rate of the shoot apex can be quite impressive. If cytokinin and auxin had been present new leaves would have been initiated, as these are necessary for leaf ignition (Lydon, 1990). As expected, the agar media performed least well; this was because the media had no additional nutrient or sugar to aid in the explants development.

## Influence of of auxin and cytokinin

The auxin 2,4-D and cytokinin BAP were added to the growth mediums to investigate any alternations in the response of the hypocotyls. The mount of callus present was assessed visually on a scale of 0 to 4 where 0 = no callus, and 4 = large callus. 2, 4-D had a callus rating of 2 where BAP did not produce any callus. However, the

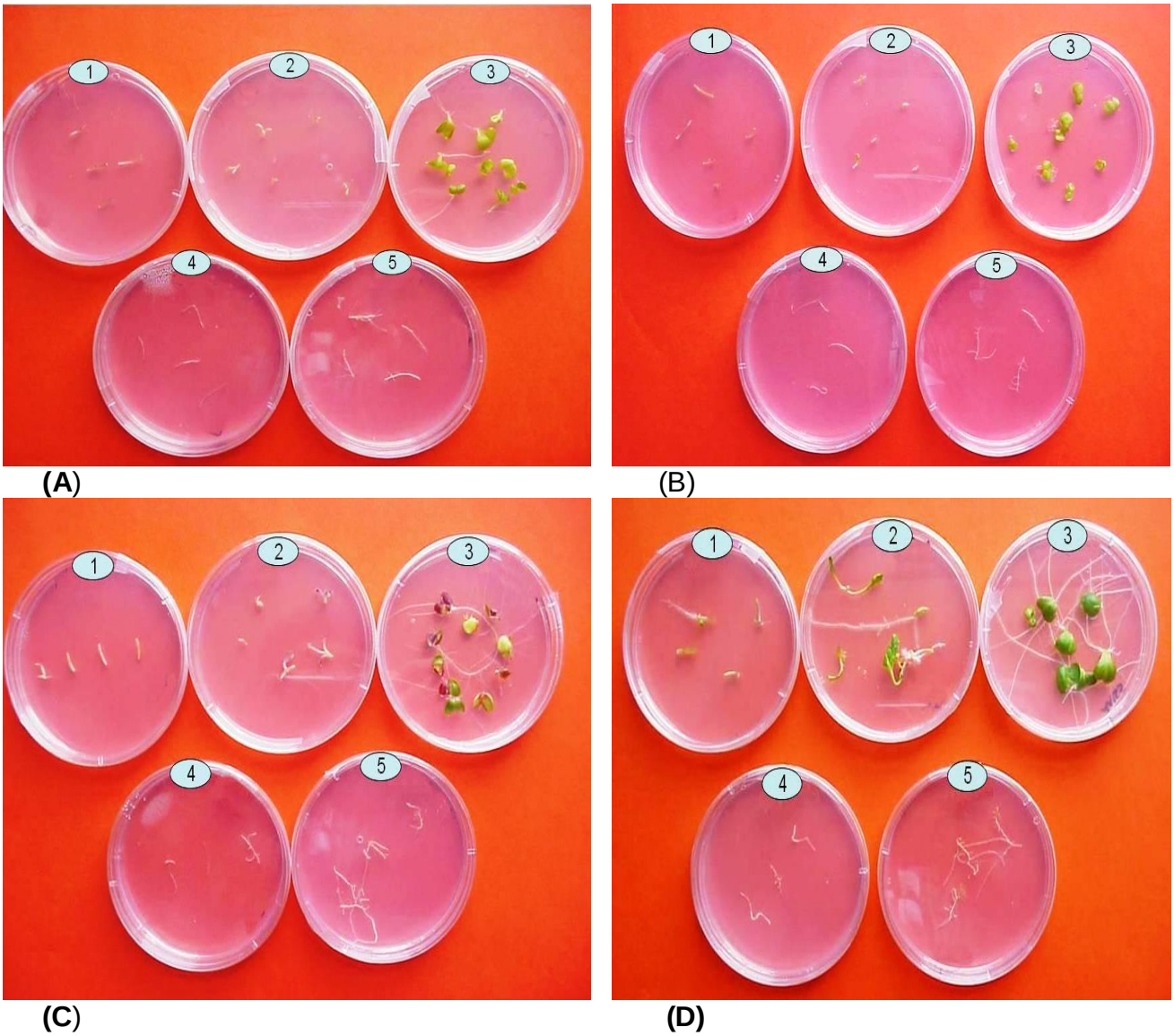


**Figure 1.** The effect of (a) agar, (b) Agar + MS, (c) Agar + Sucrose and (d) Agar + MS + Sucrose media on the growth of different explants: 1 - Hypocotyls, 2 - Shoot apex, 3 - cotyledon, 4 - Root tip, and 5 - Primary root.

combination of two hormones produced a maximum rating of 3 (Figure 3). Each cell in the callus differentiated into phloem and xylem and small plantlets were produced (Fuller and Fuller, 1995). There was no production of adventitious roots in any of the hormone treated media; this would probably be due to time. If they had been left for longer time, adventitious roots may have developed on the auxin media, the cytokinin media would not be expected to produce root, but may produced adventitious shoot (Blakesley and Thomas, 1987). Our results agree with those of Teale et al. (2008) and Faiz and Muhamed (2012), and indicated that the auxin to cytokinin ratio was decisive in the *in vitro* response of plant tissue; exposing callus cultures to a high ratios of auxin: cytokinin resulted in root formation, whereas a low ratio promoted somatic embryogenesis as auxins are known to regulate cytokinin pool size and vice versa.

The increase in length of the hypocotyls explants was due to the auxin activity as it is involved in the process of stem elongation; this would have been the case with agar plus sugar plus MS plus 2,4-D and the agar plus MS plus sugar plus 2,4-D and BAP (Table 4). The formation of

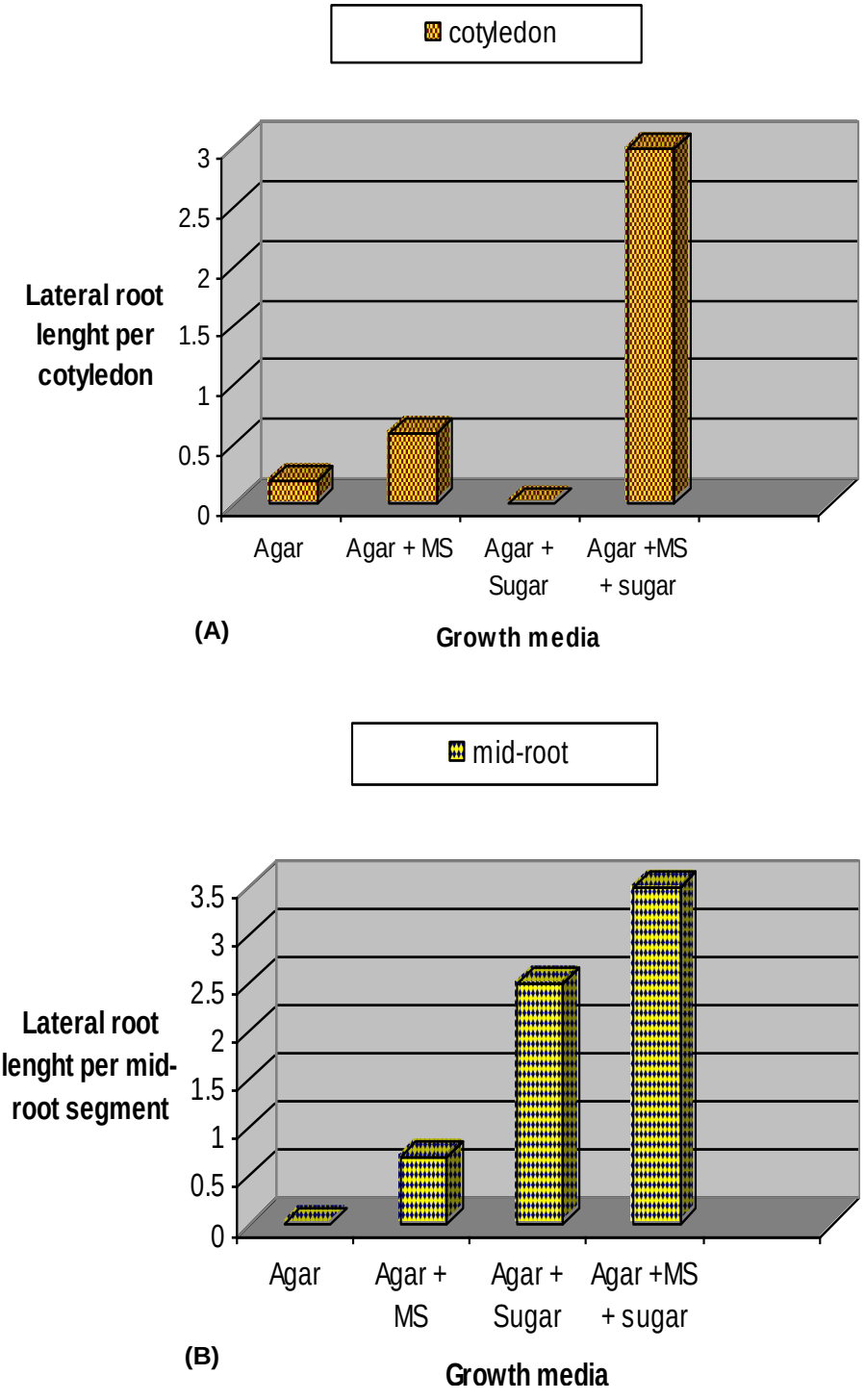


**Figure 2.** Average number of lateral root per (a) cotyledon and (b) per mid-root segment in different media after 30 days.

adventitious roots by the stem is also stimulated by auxin and inhibited by cytokinin (Forbes and Watson, 1996). Modarres and Jami (2003) and Taveira et al. (2009) indicated that MS liquid medium supplemented with 2

**Table 2.** Analysis of variance of different media component on thegrowth ofdifferent explants at different date.

| Source of variance | df | Explant | | | | |
|---|---|---|---|---|---|---|
| | | Cotyledon length | Hypocotyl length | Shoot apex length | Primary root length | Root tip length |
| Date of data | 3 | 1.312** | 0.207** | 1.10** | 0.747** | 1.59** |
| Type of media | 3 | 1.537** | 0.232** | 2.36** | 0.382** | 0.423** |
| Date of data x Type of media | 9 | 0.5235** | 0.015$^{ns}$ | 0.54** | 0.107** | 0.175** |
| Error | 32 | 0.0038 | 0.0038 | 0.01 | 0.0075 | 0.012 |

**Table 3.** Correlation between different explants under different treatments

| Explant | Root tip length | Primary root length | Shoot apex length | Hypocotyl length |
|---|---|---|---|---|
| Cotyledon length | 0.62** | 0.70** | 0.87** | 0.63** |
| Hypocotyl length | 0.51** | 0.62** | 0.66** | |
| Shoot apex length | 0.60** | 0.67** | | |
| Primary root length | 0.71** | | | |

* and ** significant difference at 5% and 1%, respectively.

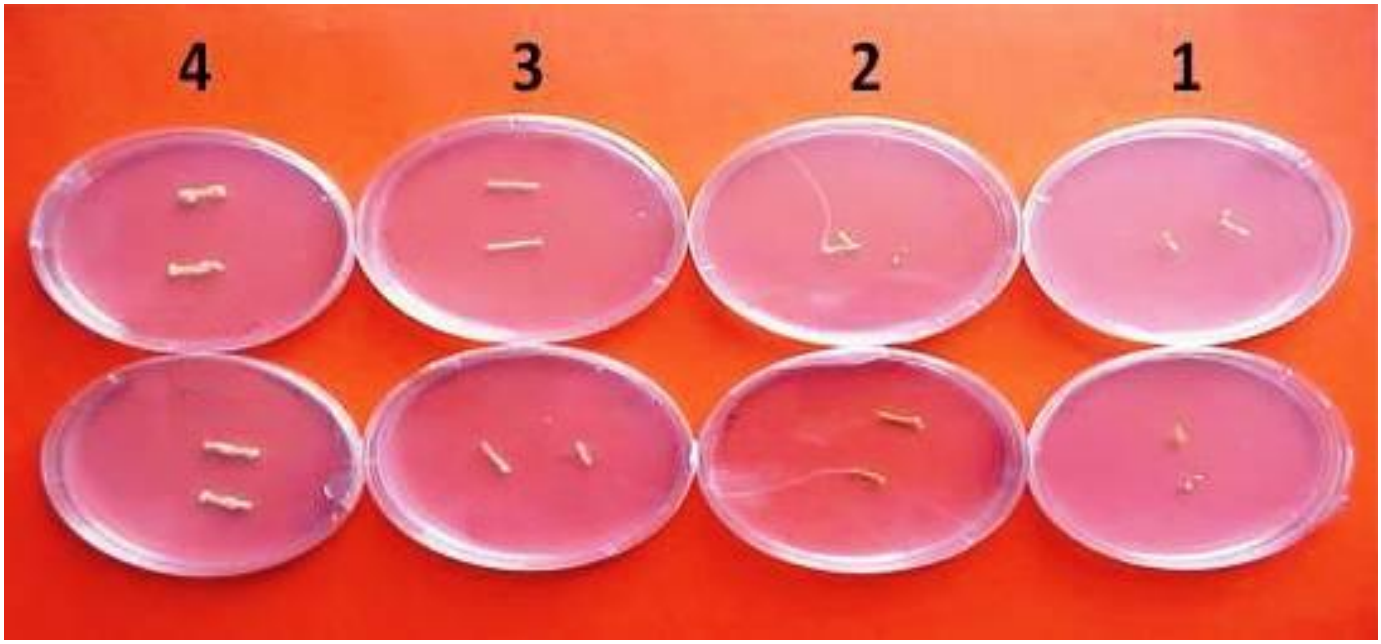


**Figure 3.** The effect of different mixture of auxin and cytokinin on the development of hypocotyl explants. 1 = A+MS+S; 2 = A+MS+S+BAP; 3 = A+MS+S+2,4-D; 4 = A+MS+S+BAP+2,4-D.

mg/L benzylaminopurine (BAP) and 0.1 mg/L naphthalene acetic acid (NAA) revealed to be the best *in vitro* condition to produce shoot and root material.

**Solid versus liquid culture**

The cultures grown in the solid media produced the

**Table 4.** The mean effect of different mixture of auxin and cytokinin on the development of hypocotyls explants.

| Explant | Days | Agar (7 g/L) + MS (4.4 g/L) + sugar (30 g/L) | Agar (7g/L) + MS (4.4 g/L) + sugar (30 g/L) + 2,4-D (0.002 g/L) | Agar (7 g/L) + MS (4.4 g/L) + sugar (30 g/L) + BAP (0.003 g/L) | Agar (7 g/ L) + MS (4.4 g/L) + sugar (30 g/L) + 2,4-D (0.002 g/L) + BAP (0.003 g/L) |
|---|---|---|---|---|---|
| Hypocotyls | 0 | 0.9 | 0.9 | 0.9 | 0.9 |
| | 10 | 1.00 | 1.3 | 1.2 | 1.3 |
| | 20 | 1.1 | 1.4 | 1.4 | 1.5 |
| | 30 | 1.2 | 1.8 | 1.6 | 2.00 |
| Average | | 1.05 | 1.35 | 1.27 | 1.42 |

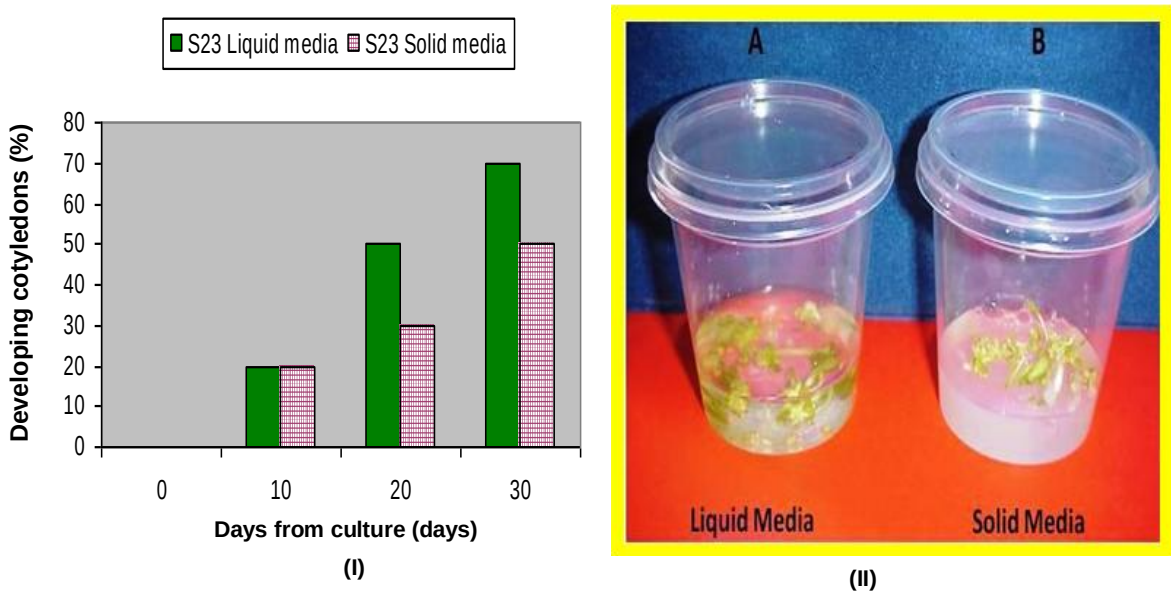


**Figure 4.** Developing cotyledon on cauliflower curd in S23 liquid and solid media after 30 days.

greatest proportion of shoot more than liquid medium (Figure 4). The growth of meristematic tissue shavings from the floret of cauliflower had better result in the liquid medium container. The movement of the rotary shaker provided vital aeration of the medium to sustain cell respiration in the liquid and also encourage the meristematic curd layer to remain separated condition of light, temperature, and aeration. The explants that were incubated in liquid medium on the shaker appeared to be healthier and greener than the explants grown on the solid medium. The explants grown on the solid medium were much slower to develop, and much smaller.

In contrast, the explants grown in liquid medium were fast to develop; they produced large plants that looked green and healthy. They had not only received the nutrients and fluid required for their development but had been prevented from drowning in the fluid by the continuous rocking motion of the platform. Kiffer et al. (1995), found that an agitated liquid medium was superior in terms of the number of reactive explants and their speed of growth to semi-solid and liquid medium. This was due to intense competition between the shoots for nutrients, which resulted in the number of good quality shoot recovered; being low. The use of liquid culture medium limited this competition effect and optimized the number of shoot recovered. When the rooting step took place in the liquid medium up to 40% of the rooted shoots recovered were hypehydric and difficult to wean. It is seemed that liquid mediums increased growth by reducing competition for nutrients, in a fashion similar to hydroponics. On the other hand, Badawi et al. (1996) showed that liquid medium did not affect the percentage of surviving cutting, but root cutting percent and shoot length decreased.

## Conclusion

It appears that *in vitro* selection for stress tolerance will continue to have its significant place in the strategy of establishing plant system with optimal stress reaction and productivity. The establishment of an efficient regeneration protocol is a pre-requisite to effective exploitation of most biotechniques. Conventional systems of morphogenesis responses can be improved by *in vitro* manipulation of determination factors. We have established callus induction and regeneration protocol for cauliflower. This protocol will pave the way for the development of *in vitro* regeneration system for cauliflower and consequently will promote the application of plant tissue culture technology in the area of selection resistance, production of artificial seeds, and genetic transformation. Finally, from the present data, it can be concluded that the cotyledons grown in the Agar+MS+Sugar were the most developed compared with plant tissues in the other growth medium. The seedling which produced from the callus induced from hypocotyl on Agar+Sugar+MS+2,4-D+BAP media were the best for cultivation, and it could be induced into a breeding program in the future for improvement the cauliflower production under drought, salt and temperature stress. S23 Liquid media are recommended for successful regeneration of cotyledon explants.

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