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In vitro Response Different Explants of Mungbean Seedlings (*Vigna radiata* L.) for Plant Growth Hormones

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Abstract

The study aimed to evaluate the response of mungbean explants (shoot tips, cotyledon leaves, hypocotyls and epicotyls) for callus induction, plant regeneration and other phenotypic responses. Different combination of Cytokinins (Kin, BA) and Auxins (IAA, NAA) employed in media Murashige and Skoog (MS), 4 weeks after culturing a significant difference were found among the explants responses to plant hormones. Maximum percentage of callus induction100% in media (T10, T11 and T12) compare to 22% at T1 media free hormones beside Epicotyls exhibit higher callusing response 68% in media supplemented with BA and NAA compare with 81.33% for Hypocotyls in media supplemented with IAA and KINT.

Keywords: mungbean, Explants, *In Vitro*, callus induction

INTRODUCTION:

Vigna radiata L. commonly known as mungbean, green gram, moong and golden gram (1). The species of mungbean moved from the genus *Phaseolus* to *Vigna* but it is often called *Phaseolus aureus* or *Phaseolus radiates* (2). Prasad *et al*. (3) stated the contribution of grain legumes nearly 15% of total pulse production. Seeds of mungbean are a rich source of protein (4) and the sprouts used as forage (5). According to Sharma and Dhanda (6), mungbean contain 4-6g of carbohydrate in addition to vitamins (A,B,C,E) which makes it popular among vegetarians. Regarding to its medical activity, it is used as anticancer due to the presence of Lectin (2). *In Vitro*, different studies were made about the response of mungbean explants for various combinations of plant hormones. To obtained undifferentiated mass of callus from different explants, or regenerations, standardize the culture media, hormones and explants combinations are required (7).

Attempts to propagate mungbean *In Vitro* made many successes in various aspects, Rao and Patil (8) regenerated plants from stressed salted callus of mungbean while Rajendiran *et al*. (9) exposed the callus to radiation stress.

The present study conducted to evaluate response of different explants initiated *In Vitro* from seedling of mungbean in the presence of various combinations of plant growth regulators.

MATERIAL AND METHODS

The current research was conducted at The Ministry of Science and Technology/ Directorate of Agricultural Research, Genetic Engineering Department, during the year 2018. Seeds of mungbean local verity were obtained from local market.

Media preparation

In general, Basal MS (10) media was used in all experiments fortified with organic component described in table (1) for germination and supplemented with various combinations of Auxins and cytokinins for callus induction media table (2) Stock solution of Auxins group were prepared by dissolving with 0.5 N of NaOH and Cytokinins with 0.1 HCl.

For seeds germination *In Vitro*, surface sterilized in aseptic condition have done by spraying the seeds with 70% ethanol for 10 second, washed with distilled water. Seeds were soaked with different concentration of Sodium hypochlorite (6%NaOCl) for different periods as it shown in table (3). After treatment, seeds were washed with distilled water trice time 5 min each. Seeds were germinated in 5 jars (10-12) seeds each 7days later, Contamination % and germination% were recorded.

Table1:Media MS with organic component ($mg.l^{-1}$)

MS salts	4400
Glycine	2.0
Myo-insitol	100
Nicotinc acid	0.5
Thiamine- HCl	0.1
Pyridoxine	0.5
Sucrose	30000

Callus induction media and culture of explants

MS media with different cytokinins concentrations (6-benzyl amino purine (BA) and Kinetin (KINT) in combination with Indole acetic (IAA) or Naphthalene acetic acid (NAA) at different concentrations as it shown in table (2). pH of all media adjusted to 5.75±2 and solidified with ($7g.l^{-1}$) agar as gelling agents and autoclaved at 121°C for 15 minutes. Shoot tips, cotyledon leaves, epicotyls and hypocotyls were excised from seedling of 7 days age (fig 1-A) mungbean. All explants were transferred to callus induction media. 4 weeks later, data recorded. All *In Vitro* cultures were conducted in aseptic condition inside laminar air flow cabinet. The experiments designed in completely randomized (C.R.D), and means were compared at least (L.S.D) at $P \leq 0.05$ level. DATA analysis using GenStat software program.

Table 2: Different plant growth regulators and their cods tested for different explants

Code	Growth regulators mg.l^{-1}	References	Type of explants
T1	MS free hormones (control)	__	Cotyledon leaves, Epicotyls, Hypocotyls, Shoot tips
T2	BA(0.5)	(3)	Cotyledonary nodes
		(10)	Cotyledons
		(11)	Cotyledons
		(12)	Cotyledons
		(13)	leaves
T3	NAA(0.1)	(12)	Cotyledons
T4	BA(0.1)NAA(0.2)	__	Cotyledon leaves, Epicotyls, Hypocotyls, Shoot tips
T5	BA(0.5)NAA(2)	(12)	Cotyledons
T6	BA(1)NAA(1)	(11)	Cotyledons
T7	BA(2) NAA(0.5)	(11)	Cotyledons
T8	IAA(0.5)KINT(0.5)	(14)	Cotyledon leaves, Hypocotyls, Epicotyls
T9	IAA(1) KINT(1)	(15)	Shoot tips, Cotyledon leaves, Hypocotyls
T10	IAA(2) KINT(2)		
T11	IAA(4) KINT(4)	__	Cotyledon leaves, Epicotyls, Hypocotyls, Shoot tips
T12	IAA(6) KINT(6)	__	Cotyledon leaves, Epicotyls, Hypocotyls, Shoot tips

Table 3: seeds contamination % of green gram

NaOCl% (v/v)	% contamination
Ethanol 70% 10 sec	100
1% NaOCl 10 min	80.00
1% NaOCl 15 min	50.00
2% NaOCl 10 min	16.31
2% NaOCl 15 min	0.00
	LSD $_{0.05}$ 1.42

Table 4: callus induction % in MS media and various hormones treatments.

Code of treatments	Hormonal treatment mg.l^{-1}	% callus induction
T1	MS free hormones (control)	22.00
T2	BA(0.5)	56.00
T3	NAA(0.1)	47.00
T4	BA(0.1)NAA(0.2)	54.00
T5	BA(0.5)NAA(2)	83.00
T6	BA(1)NAA(1)	60.00
T7	BA(2) NAA(0.5)	85.00
LSD $_{0.05}$ 5.99		
T8	IAA(0.5) KINT(0.5)	52.00
T9	IAA(1) KINT(1)	88.00
T10	IAA(2) KINT(2)	100.00
T11	IAA(4) KINT(4)	100.00
T12	IAA(6) KINT(6)	100.00
LSD $_{0.05}$ 3.53		

RESULTS AND DISCUSSION

Based on data summarized in table (3) highest contamination 100% when seeds treated with 70% ethanol for 10 sec compare with no infection in 2% NaOCl 15 min. Also significant differences were found among the time and concentration of NaOCl however, in all cases increasing time and concentration of the NaOCl affected positively on seeds contamination. Surface sterilization is a critical stage and challenging step that determines the success of culture establishment in the next stage.

In our experiment, ethanol and sodium hypochlorite proved their efficiency with no mortality or damage seed viability. Hypochlorous acid is (HOCl) is formed through the reaction of water with NaOCl which in turn release Cl (16). Several authors tend to use NaOCl and ethanol in their *in vitro* experiment. Sen *et al*.(17) used ethanol, antibiotics, fungicides and mercuric chlorite ($HgCl_2$), dispute the risk of the last sterile which in turn needs special handling (18,19).

For the response of explants to callus induction, in general highest callus induction obtained 100% in media T10,T11,T12 (IAA(2) KINT(2), IAA(4) KINT(4), IAA(6) KINT(6) mg.l^{-1} respectively (Table, 4), while media T1 (MS free hormones) gave lowest 22% response. Furthermore, in the presence of different combinations from BA and NAA, Epicotyls was superior to give 68.57 % while Hypocotyls 81.33 in presence of different combinations of IAA and KINT (Table 5, 6) respectively.

Plant hormones are widely used *In Vitro*. However, response toward callogenesis, organogenesis is varied among species, families, and plants or even among explants from the same plant. In our research, callus was raised from all explants of green gram when exposed to different combination of hormone even in control medium (T1) free hormones; this may be due to the high endogenous levels of Auxin in the explants which control the cell division and callus growth (18). Concerning to the nature of callus, compact callus only was observed in (T5,T6,T7) unlike friable callus with shoot regeneration observed in media (T2,T10, T11,T12) the results are agree with (8,20,21,22). Moreover; callus with roots initiated from shoots and epicotyls explants were observed on media IAA (4) KINT (4) mg.l^{-1} (Fig 1-B) and friable callus initiated from epicotyls and hypocotyls on media (BA(0.5) NAA(2) mg.l^{-1} (Fig 1-C), and in both cases no plants regeneration were found. Several studies reported response of explants which derived from seedling of legume species found organogenesis,callogenesis directly or via callus (23,24,25).

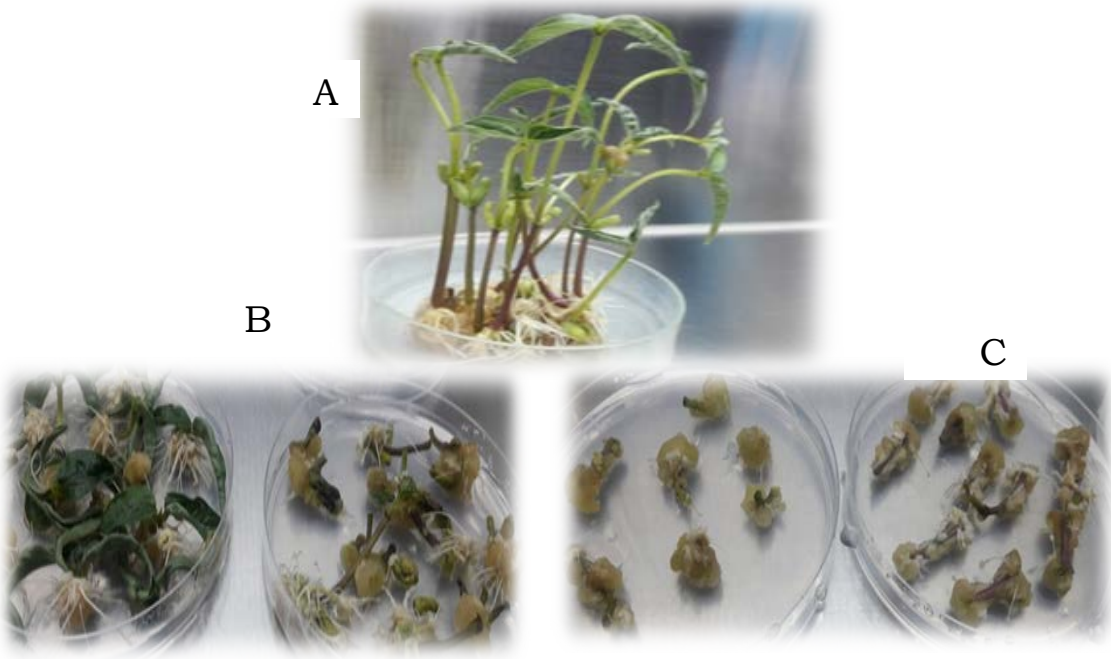

Fig 1:A seedling of green gram age 10 days. (B) callus with roots initiated from shoots and epicotyls explants on media (IAA(4) KINT(4) mg.l^{-1}).(C) callus initiated from epicotyls and hypocotyls on media (BA(0.5)NAA(2) mg.l^{-1})

Table 5: Response of explants for callus induction in presence of different combinations of BA and NAA.

Type of explants	% callus induction
Shoot tips	49.71
Cotyledon leaves	52.00
Epicotyls	68.57
Hypocotyls	62.29
LSD $_{0.05}$	**4.53**

Table 6: Response of explants for callus induction in presence of different combinations of IAA and KINT.

Shoot tips	74.00
Cotyledon leaves	74.00
Epicotyls	78.67
Hypocotyls	81.33
LSD $_{0.05}$	**2.88**

CONCLUSION

In conclusion, green gram explants have flexibility responses for different hormones combinations. Hence further studies required for detection somaclonal variation among plants regenerated in vitro and field evaluation for yield, morphological and genetic traits for plants produced in vitro via either directly or indirectly via callus.

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