EFFECT OF EXPLANT AND GENOTYPE ON CALLUS CULTURE AND REGENERATION IN ONION (*ALLIUM CEPA* L.)

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Abstract

The present investigation explores the possibility of developing *in vitro* system which could have wider application such as germplasm conservation micropropagation, production of virus/disease free materials. Plant regeneration system evolved in this study may help researchers to improve these crops. Onion is one of the most important vegetable crop grown throughout the world. Three genotypes of onion viz., B-780, N-2-4-1 and Hisar-2 along with three explants i.e., shoot tip, root tip and seeds were used to study the effect of genotype and explant on indirect organogenesis in onion. *In vitro* grown axenic seedlings were used as the explant source. It was observed that on an overall basis MS medium supplemented with 2,4-D 0.5mg/l was optimum for maximum callus formation in all the three explants studied. Among the genotypes tested, B-780 was found to be best genotype giving good response and in explants shoot tip was adjudged best on the basis of its callus formation ability.

Keywords: *Allium cepa*, genotype, explant, callus culture, regeneration

Soğanda *(Allium cepa* L.) Eksplant Tipi ve Genotipin Kallus Kültürü ve Regenerasyon Üzerine Etkileri

Özet

Bu çalışmada germplazm muhafazası, çoğaltma, hastalık ve zararlılardan ari üretim için geliştirilen *in vitro* tekniklerinin soğanda kullanım olanakları araştırılmıştır. Araştırmada geliştirilen bitki regenerasyon sistemi, dünyada yetiştirilen en önemli ürünler arasında yer almakta olan bu türün geliştirilmesinde araştırıcılara yardımcı olacaktır. Çalışmada 3 soğan genotipi (B-780, N-2-4-1 and Hisar-2) ve sürgün ucu, kök ucu ve tohum olmak üzere 3 farklı eksplantın direkt ve indirekt organogenesis üzerine etkisi araştırılmıştır. *In vitro* eksplant kaynağı olarak axenic fideler kullanılmıştır. Her üç eksplantta da maksimum kallus oluşumu için MS ortamına 0.5 mg/l 2,4-D eklenmesi uygulaması en iyi sonucu vermiştir. Denenen gentotipler arasında B-780, kallus oluşumu açısından en iyi genotip ve sürgün ucu ise en uygun eksplant tipi olarak belirlenmiştir.

Anahtar Kelimeler: *Allium cepa*, genotip, eksplant, kallus kültürü, regeneration

1. Introduction

Onion (*Allium cepa* L.), a member of *Alliaceae* family, is one of the most important vegetable crop grown throughout the world for its medicinal and cuisine value. No Indian kitchen is complete without this vegetable crop. India ranks second to China in area (4.26 lakh ha.) and production (54.84 lakh tonnes) with an average productivity of 12.87 t/ha (Anonymous, 2002). Besides this, about 90% of vegetable export form India comprises mainly of onion. A lot of work on conventional methods of breeding and production has been done in India but the *in vitro* studies on Indian genotypes are in rudimentary stage. Except a few reports on garlic (Bhojwani, 1980; Koul et al., 1994) not much work has been done in this crop. Tissue culture provides an affordable alternative for propagation of elite and rare material, development of homozygous lines through anther/ovary/ovule culture and, thereby, jettisoning the breeding programme since onion is an outbreeder with a 'two years per generation cycle' crop. Moreover, an efficient and reproducible protocol is important for development of any transformation protocol. Keeping in view all the above facts, an experiment was conducted to study the effect of explant and genotype on callus culture and regeneration in onion.

2. Materials and Methods

Three genotypes of onion viz.,

Baswant 780, N-2-4-1 and Hisar 2 were used in the present investigations. Onion seeds were surface sterilized by immersing them in sterile water containing two drops of Tween 20 per 100 ml and 0.3% Benlate / Bavistin for 15-20 minutes with continuous agitation followed by 5-10 washings with sterile tap water in order to remove the fungicides and other residues. They were then surface disinfested for second time by passage through 70% alcohol for 2 minutes, 0.1% $HgCl_2$ or 2% NaOCl for 15-20 min and then rinsed in sterile deionised water 4-5 times. Seeds were cultured on simple MS (Murashige and Skoog's 1962) medium to develop seedlings. After 10-15 days of seedling development, *In vitro* grown axenic seedlings were used as the explant source. Explants used in the present study were shoot tip, root tip and seeds. The experiment was laid in factorial randomized block design with three replications and the analysis was carried out using MSTAT.

2. Results and Discussion

2.1. Callus formation in shoot tip

In shoot tip explant, best results were observed when 2,4-D 0.5 mg/l was used in all the three genotypes. Increase in the concentration of 2,4-D had a negative impact on callus initiation and proliferation where increase in concentration of 2,4-D (from 0.5 mg/l to 5.0 mg/l) decreased callus initiation efficiency of explants from over 74.1% to 17.2% (Table 1). However, supplementation of casein hydrolysate 300 mg/l with C1 medium enhanced callus initiation (77.3%) up to some extent in all the three genotypes. Use of casein hydrolysate with higher concentration of 2,4-D (2.0 mg/l) did not have any positive effect on callusing efficiency of shoot tip explants. TDZ (Thidiazuron), a urea compound with cytokinin like activity, did not show any significant effect on callusing when used in combination with 2,4-D. Picloram 0.5 also exhibited higher callus induction behaviour in all the three cultivars. Increasing concentrations of picloram further (0.5-5.0 mg/l) reduced callus formation and proliferation in shoot tip explant of all the three genotypes of onion. Also when picloram was tried in combination with optimum concentration of 2,4-D i.e., 0.5 mg/l, decrease in callusing response of all the cultivars was observed. Based on the results, C5 medium (2,4-D 0.5 + Casein hydrolysate 300 mg/l) was adjudged best among all the media tried for callus initiation and proliferation for shoot tip explants of the three studied cultivars of onion with an overall mean percent callus formation of 77.3%. Based on statistical analysis it was observed that cv. B-780 was significantly superior in callus formation (42.0%) over cv Hisar-2 (36.8%) and N-2-4-1 (37.9%) which were significantly at par with each other.

Different cytokinins i.e., kinetin, BA and 2iP were also tried along with picloram for initiation of callus in shoot tip explant (Table 2). When picloram was used at 2.0 mg/l along with kinetin at two different concentrations i.e., 1.0 and 2.0 mg/l (C1 & C2), the callus induction was highest in both with no apparent significant differences within the cultivars. BAP 0.5 mg/l along with picloram at 0.1 mg/l exhibited highest percentage of callus induction (78.6%) where cultivar N-2-4-1 showed maximum efficiency (83%) followed by Hisar-2 and B-780 (77.5% and 75.3%, respectively). When a combination of one auxin and a cytokinin i.e., 2iP and picloram were used, it was observed that 2iP 0.5 mg/l (C10) along with picloram 0.1 mg/l induced highest callus formation in B-780 (81.4%) followed by N-2-4-1 (74.5%) and Hisar-2 (72.9%). Since medium C1, C2, C6 and C10 were significantly at par with in their efficiency for callus induction from shoot tip explant, they were exploited for plant regeneration studies.

Based on statistical analysis, it was observed that all the three genotypes differed significantly in their ability for callus formation from shoot tip explant. B-780 was significantly superior in callus formation (48.8%) from shoot tip explant over cv N-2-4-1 (46.8%) which in turn was significantly superior over cv. Hisar-2 (41.9%).

Table 1: Effect of auxins on per cent callus induction in shoot tip explant of onion

Sym	Constituents	Conc. (mg/l)	B-780	Hisar-2	N-2-4-1	Mean
C1	2,4-D	0.5	79.4±2.41	67.6±1.57	75.3±2.39	74.1±2.04ab
C4	2,4-D	5.0	15.0±1.73	9.3±0.62	27.3±3.68	17.2±2.91ij
C5	2,4-D + Casein Hyrolysate	0.5+300	82.3±0.47	72.9±2.09	76.7±1.50	77.3±1.55a
C7	2,4-D + Casein Hyrolysate	2.0+300	52.8±0.75	54.2±2.31	56.5±0.90	54.5±0.92c
C9	2,4-D + TDZ	0.5+1.0	13.3±1.76	14.5±0.50	13.3±1.90	13.7±0.78jk
C14	Picloram	0.5	72.0±1.73	76.1±0.87	70.5±1.27	72.8±1.06b
C17	Picloram	5.0	49.7±5.90	17.7±1.07	25.0±3.40	30.8±5.22g
Mean			42.0±2.86a	36.8±2.51b	37.9±2.53b	
CD (0.05) Variety*Medium 4.23			Within treatment 3.45		Among genotypes 1.24	

Table 2: Effect of auxin and cytokinin combination on per cent callus induction in shoot tip explant of onion

Sym.	Constituents	Conc. (mg/l)	B-780	Hisar-2	N-2-4-1	Mean
C1	Picloram + Kinetin	2.0 + 1.0	74.3±4.07	74.4±2.03	85.4±1.63	78.0±2.30a
C2	Picloram + Kinetin	2.0 + 2.0	85.3± 3.18	66.6±2.07	72.6±0.54	74.9±2.96a
C6	Picloram+ BA	0.1 + 0.5	75.3±2.39	77.5±1.50	83.0±1.93	78.6±1.51a
C10	Picloram+ 2iP	0.1 + 0.5	81.4±0.72	72.9±1.65	74.5±1.58	76.3±1.47a
Mean			48.8±3.93a	41.9±3.74c	46.8±3.94b	
CD (0.05)		Variety*Medium 5.03 Within treatment 3.55 Among genotypes 1.93				

2.2. Callus formation in root tip

From *in vitro* grown onion seedlings, roots were excised to study their callusing response. Based on overall mean of all the three genotypes in response to the medium and combinations used, medium comprising 2,4-D 0.5 + picloram 2.0 was found to be significantly superior (83.6%) over other media combinations (Table 3). This was followed by 2,4-D 0.5 + casein hydrolysate 300 mg/l and 2iP 0.5 + picloram 0.5 with an overall mean callus induction rate of 81.7% and 79.5%, respectively. On the basis of overall performance of all the genotypes of onion viz., B-780, N-2-4-1 and Hisar-2, var. B-780 and N-2-4-1 were significantly at par in their callus induction efficiency whereas Hisar-2 showed significant difference in callus induction frequency. Variety x medium interactions were found to be significant among the medium and varieties.

In var. B-780, three mediums i.e., C11 (2,4-D 0.5 + Picloram 2.0), C5 (2,4-D 0.5 + casein hydrolysate 300 mg/l) and C19 (2iP 0.5 + Picloram 0.5) were found to be significantly at par in exhibiting maximum callus induction in root tip of onion with frequency of 83.7%, 82.3% and 80.3%, respectively. Medium C16 (Picloram 5.0) showed the least amount of callus induction (23.9%) among all the media tested.

Medium C11 (2,4-D 0.5 + Picloram 2.0) and medium C5 (2,4-D 0.5 + casein hydrolysate 300 mg/l) showed the maximum amount of callus induction in variety Hisar-2. This was followed by medium C9 (2,4-D 0.5 + picloram 0.5) and C19 (2iP 0.5 + Picloram 0.5) with the induction frequency of 78.3% and 75.1%, respectively. Minimum amount of callus induction was reported in C16 (Picloram 5.0) with callus induction formation of 21.3%.

In variety N-2-4-1, maximum callus formation frequency was observed in medium C11 (2,4-D 0.5 + Picloram 2.0) with an induction frequency of 84.3% and was found to be significantly at par with medium comprising C19 (2iP 0.5 + picloram 0.5), C5 (2,4-D 0.5 + casein hydrolysate 300) and C1 (2,4-D 0.5) with the callus induction frequency of 83.0%, 82.7% and 81.1%, respectively. Least amount of callus formation was reported in medium C8 comprising 2, 4-D 2.0 + Glutamine 500 mg/l.

Table 3: Effect of different media combinations on per cent callus induction in root tip explant of onion

Sym	Constituents	Conc. (mg/l)	B-780	Hisar-2	N-2-4-1	Mean
C1	2,4-D	0.5	78.4±2.03	72.3±1.45	81.1±2.08	77.3±1.59cd
C5	2,4-D+Casein Hyrolysate	0.5+300	82.3±1.45	80.0±2.89	82.7±1.85	81.7±1.15ab
C8	2,4-D+Glutamine	2.0+500	31.7±2.08	23.8±2.00	25.4±2.52	26.9±1.63m
C9	2,4-D+Picloram	0.5+0.5	72.1±2.85	78.3±1.76	75.1±1.73	75.2±1.39de
C11	2,4-D+Picloram	0.5+2.0	83.7±2.60	82.7±1.20	84.3±2.33	83.6±1.09a
C16	Picloram	5.0	23.8±2.31	21.3±2.18	26.8±1.15	23.9±1.17m
C19	2iP+Picloram	0.5+0.5	80.3±0.88	75.1±2.08	83.0±1.15	79.5±1.38bc
Mean			59.4±2.45a	57.9±2.49b	59.8±2.47a	
CD (0.05)	Variety*Medium 5.54 Within treatment 3.20				Among genotypes 1.24	

2.3. Callus formation in seed explant

Eighteen media comprising of MS basal salts along with different auxin and cytokinin concentrations were used to test their efficacy for callus culture in seed explant of three onion genotypes. Out of these media, media C13 comprising 2iP 0.5 and picloram 0.1 was found to be best with an overall mean callus induction efficiency of 83.1% and was found to be significantly superior over all other media for all the three genotypes (Table 4). Media C18 comprising picloram 2.0 and kinetin 2.0 was the next best media for overall callus induction mean (79.27%) in these genotypes.

In terms of genotypic response to callus formation, var. B-780 (54.55%) was found to be superior and was at par with var. N-2-4-1 (53.59%). Both the varieties were significantly superior to var. Hisar-2 (48.45%). Genotype X medium interactions showed that medium C13 supplemented with 2iP 0.5 and picloram 0.1 was best in all the three genotypes with the callus formation frequency of 86.1%, 79.0% and 84.0%, respectively.

In onion, MS supplemented with 2, 4-D 0.5 mg/l was found optimum for maximum callus formation in shoot tip, root tip and seed. Decreasing or increasing the concentration of 2,4-D led to decline in callus formation. Similar observations have been reported (Dunstan and Short, 1978; Novak, 1980) who demonstrated that using 2, 4-D beyond certain level inhibited callus regeneration or resulted in loss of morphogenic capability and karyotypic instability in *A. sativum L. callus*. Zheng *et al.,* (1998) suggested that callus induction and propagation is largely determined by the concentrations of 2,4-D. Picloram is superior to 2, 4-D for continued maintenance and friability of callus and subsequent regeneration of plants, and these advantages should provide greater flexibility in developing tissue culture methods for the improvement of onion (Phillips and Luteyn, 1983). Picloram was used at varying concentration from 0.5-5.0 mg/l to induce callus formation in shoot tip, root and seed explant of onion. Picloram 0.5 mg/l in both shoot tip and root tip whereas in seed, picloram at 1.0 mg/l resulted in higher amount of callus formation. Subsequent increase in picloram concentration from 1-5 mg/l resulted in significant decrease in callus induction frequency.The results are in conformity with Phillips and Collins (1983) who reported that picloram induces callus production from onion tissue as efficiently as 2, 4-D).

Different combinations of picloram with cytokinins like kinetin, BA and 2iP were tried for callus induction in shoot tip, root and seed explant of onion. Picloram 2.0 mg/l when used in combination with 1.0 mg/l kinetin resulted in maximum callus induction in shoot tip and seed explant and was at par with kinetin 2.0 mg/l for shoot tip callus induction. Increase in BA concentration from 0.05mg/l to 0.1 mg/l led to sharp increase in callus formation (78.60%). Further increase in picloram concentration (0.1-0.5 mg/l) did not yield significant increase in callus formation efficiency. The results are in contrast to Phillips and Luteyn (1983) who developed

Table 4: Studies on the effect of different media combinations on per cent callus induction in seed explant of onion

Sym	Constituents	Conc. (mg/l)	B-780	Hisar-2	N-2-4-1	Mean
C2	2,4-D	0.5	78.0±1.76	69.3±0.88	75.0±2.08	74.1±1.52c
C4	2,4-D	2.0	22.3±1.53	16.8±1.53	19.4±0.58	19.5±1.08l
C10	Picloram	1.0	80.1±0.57	70.0±0.57	75.1±1.20	75.1±1.50c
C13	2iP+Picloram	0.5+0.1	86.1±1.85	79.0±0.57	84.0±1.52	83.1±1.30a
C18	Picloram+Kinetin	2.0+2.0	80.2±0.88	75.3±1.20	82.3±0.88	79.3±1.12b
Mean			54.6±2.57a	48.5±2.56b	53.6±2.55a	
CD (0.05)		Variety*Medium 4.56 Within treatment 2.64 Among genotypes 1.07				

high frequency of somatic embryos on media containing picloram (0.75mg/l) and 1.5-2.0mg/l BA. A combination of 2iP with picloram (0.1-1.0 mg/l) produced variable callus induction response in shoot tip explant of onion. Maximum callus formation efficiency in shoot tip , seed and root tip was observed when 2iP 0.5mg/l was used in combination with picloram 0.1mg/l. Increased picloram concentration decreased the callus induction. Use of 2,4-D (Koch *et al.,* 1995) and supplementation with picloram and 2iP (Myers and Simon, 1998) was used for friable callus formation in onion and garlic.

The present study suggests that picloram/ 2, 4-D is required at lower concentration (0.5mg/l) for callus induction in onion based on different explants response on 2, 4-D/picloram containing media.

Media additives such as casein hydrolysate and Glutamine have profound effect on improving callus type (embryogenic) and further regeneration of these cultures. These are frequently used in monocot callus cultures for maintaining embryogenic callus cultures and obtaining regeneration the long term cultures. Monocot callus cultures usually have embryogenic as well as non-embryogenic callus. We observed that addition of these two, highly improved the callus type source; creamish yellow callus was obtained which readily formed embryogenic suspension where globular other stages of embryo developed were observed under microscope.

In case of onion shoot tip explants, use of casein hydrolysate 300 mg/l along with 2, 4-D at 0.5 mg/l significantly increased the per cent callus formation (77.3%). In seed explant, use of casein hydrolysate at 300 mg/l alongwith 2, 4-D 0.5 mg/l and 2.0 mg/l had no significant edge over callus formation frequency in comparison to 2, 4-D 0.5 when used alone. When casein hydrolysate was used along with 2, 4-D 0.5 mg/l for induction of callus in root tip explant, significant increase in callus formation (81.7%) was observed. Use of glutamine did not improve the callus formation frequency in any explant of onion. Addition of casein hydrolysate to the culture medium improved the callus frequency. Similar results were found in the study conducted earlier by Aftab *et al*., (1996) and Zheng *et al*., (1998)

2.4. Genotype specificity

Onion genotypes exhibited significant genotypic difference in response to callus formation and multiple shoot formation from different explants. On an overall basis, var. B-780 was found to be significantly superior over other two varieties. Genotypic difference in the regeneration of *Allium* have also been reported by other researchers (Barandiaran *et al.,*1999; Zheng *et al.,* 1998). Tanikawa *et al*., (1998) reported significant differences among cultivars regarding plant regeneration efficiency. In terms of suitability of explant for micropropagation, shoot tip was found best for callus formation as compared to root tip and seed explant.

2.5. Callus regeneration

Callus obtained from different explants of onion was cultured on shoot regeneration media for plantlet regeneration.

It is generally reported in monocots that the callus obtained when cultured on MS basal media leads to shoot and root formation But in our experiments, when callus obtained from different explants with different medium combinations was cultured on MS basal medium, profuse rooting and formation of albino shoots was observed (Table 5).

When kinetin 2.0 was used as shoot regeneration medium, shoot formation (5-8 shoots per piece of callus) was obtained in callus obtained from shoot tip explant of onion with picloram + kinetin combination. When BA was used, dark green callus formation was observed in callus obtained from picloram along with 2,4-D, 2iP or BA combinations. But this dark green callus was unable to plantlet regeneration even after two months of culture. TDZ (Thidiazuron) led to formation of green callus in calli obtained from picloram + kinetin and picloram + 2,4-D in onion but no plantlet regeneration was evident. When NAA 0.5 + kinetin 2.0 were used in combination, shoot formation (3-4 shoots per gram of callus) was observed in calli obtained from picloram + kinetin combination whereas albino shoot formation was observed in picloram + 2, 4-D and 2iP + picloram combinations. 2iP 0.5 when used alone resulted in shoot regeneration from calli obtained from 2iP + picloram combination after two months of culture on regeneration media whereas BA (2.0 & 5.0) when used at higher concentrations, lead to albino shoot formation in some calli. Kinetin 5.0 lead to shoot formation (4-5) in calli obtained from picloram + kinetin combination. Higher concentration of kinetin did not have any effect on plantlet regeneration.

Plant growth regulators play an essential role in *in vitro* culture in monocots such as cereal crops, the addition of cytokinin can be significant for plant regeneration (Bhaskaran and Smith, 1990). In *Allium* tissue culture, MS (Murashige and Skoog, 1962) or BDS (Gamborg's BS modified medium) supplemented with a cytokinin is normally used for plant regeneration, but auxins are not so important as cytokinins for regeneration (Van der valk *et al.,* 1992; Wang and Debergh, 1995).

In the present study several cytokinins such as BA, Kinetin, TDZ and 2iP were used for inducing shoot formation in callus cultures. Kinetin 1.0, 2.0 and 5.0 mg/l was able to induce shoots in callus obtained from Picloram + Kinetin combination. Increase in kinetin concentration to 10mg/l did not elicit any response. The results are in consonance with the findings of Mohammed Yasseen and Splitstoesser, (1992) and Saker and Sawahel (1998) who reported shoot regeneration on MS medium containing Kinetin. NAA alone did not exhibit any response but when NAA (0.5 mg/l) was used in combination with kinetin (2.0 mg/l), shoot formation was observed. In the rest calli, albino shoot formation response was observed. This is in contrast to Zheng *et al.,* (1999) who reported shoot and root formation on MS basal medium but in conformity with Wang and Debergh (1995)

Table 5: Studies on plant regeneration from callus derived from different explants of onion

Code	PGR	Conc. (mg/l)	Callus obtained from combinations			
			Picloram + Kinetin	Picloram+ 2,4-D	2iP + Picloram	Piclora+ BA
C1	MS basal	-	RF	RF	RF	RF
C3	Kinetin	2.0	$^{O}SF^{1}$	RF	RF	RF
C5	BA	1.0	DG	DG	DG	DG
C6	TDZ	0.5	DG	DG	NR	NR
C9	NAA+Kinetin	0.5+2.0	$^{O}SF^{2}$	ASF	ASF	RF
C10	2ip	0.5	NR	NR	$^{O}SF^{4}$	NR
C11	BA	2.0	ASF	NR	NR	ASF
C12	BA	5.0	NR	NR	NR	ASF
C13	Kinetin	5.0	$^{O}SF^{3}$	RF	NR	NR

ASF: Albino Shoot formation , SF: Shoot formation, DG: Dark Green Callus, RF : Root Formation NR: No response
1= 5-8 shoots per piece of callus 2= 3-4 shoots per piece of callus
3= 4-5 shoots per piece of callus 4= 4-5 shoots per piece of callus

who noted that plant regeneration in *A. porrum* was clearly dependent upon the presence of cytokinin while on growth regulator free medium shoot regeneration was hardly ever seen.

Addition of BA in the medium led to the formation of dark green callus but did not support shoot formation whereas TDZ induced dark green callus in onion derived callus. Plantlet regeneration was obtained after 55-60 days of culture. Other combinations did not promote any shoot induction. 2iP gave delayed response and shoot regeneration was recorded after 60-65 days in calli obtained from 2iP + picloram combination.

2.6. Root initiation

In vitro regenerated plantlets were shifted to the MS medium alone or along with different hormone concentrations to study their effect on root initiation. It was observed that MS (half strength) led to root formation in all the three genotypes with 10-12 roots per plant and the percentage of plantlets rooted varied from 59.8% (N-2-4-1) to 69.0% (B-780) (Table 6). The root quality was very good and the roots formed were elongated and thick in texture. When MS basal (full strength) medium was used, about 15-20 roots per seedling were obtained and the percentage of plantlets producing roots ranged from 90.5% (Hisar-2) to 93.0% (B-780). The root quality was excellent with elongated and thick root formation whereas when MS basal medium when used with either IAA 0.5 or NAA 0.5, the root quality was poor. Thick textured and stunted roots were formed. The number of roots formed varied from 6-10 in MS medium supplemented with IAA 0.5 and 5-8 roots were formed in MS medium comprising of NAA 0.5. The percentage of rooted plantlets varied from 76.3% (Hisar-2) to 89.7% (B-780) in the former media whereas in the latter medium the percentage rooted plantlets varied from 62.0% (Hisar-2) to 68.0% (B-780). No significant overall differences among the genotypes were observed for their root formation ability among individual mediums.

It was observed that use of MS basal media without any growth regulators led to the formation of roots with excellent quality in onion. The results are in accordance with Zhang *et al.,* (1995).

2.7. Plant Establishment

Callus regenerated plantlets from onion were evaluated for their survival percentage and morphological variation, if any upon subsequent transfer to the potted soil conditions (Table 7). It was observed that the survival percentage of B-780 (85.0%) was more than the cvs. N-2-4-1 (68.0%) and Hisar-2 (77.3%). The *in vitro* plants were kept in the culture room for 15-20 days for hardening at 25±2°C and were then subsequently transferred to the potted soil. When transferred to the pot conditions, the mortality of plants increased due to higher temperature and the overall survival percent in B-780, N-2-4-1 and Hisar-2 was 40%, 56% and 63.6%, respectively. When the plants were studied for morphological variations, no significant morphological variations were observed except in B-780 where some plants (5-10%) were obtained with giant and abnormal leaves.

To conclude, it can be said that a complete protocol for *in vitro* regeneration

Table 6: Effect of different growth regulators on root initiation in *in vitro* grown onion plantlets

Medium	PGR	Conc.	No. of roots	B-780	Hisar-2	N-2-4-1	Root Quality
½ MS	-	-	10-12	69.0±4.67	59.8±2.89	68.0±3.67	+++
MS	-	-	15-20	93.0±3.79	91.0±2.08	90.5±1.38	++++
MS	IAA	0.5	6-10	89.7±4.67	82.0±3.51	76.3±3.67	++
MS	NAA	0.5	5-8	68.0±1.15	64.5±3.97	62.0±8.08	++
CD (0.05)			9.54	10.3	8.8		
Interaction		9.09					

+: Poor ++: Good +++: Very Good ++++: Excellent

Table 7: Evaluation of *in vitro* grown onion plantlets after transfer to pot conditions

Genotype	% age survival during hardening stage	Overall survival	Morphological abnormality
B-780	85.0	40.0	5-10%
Hisar2	77.3	63.6	-
N-2-4-1	68.0	56.0	-

of onion through indirect organogenesis has been developed. It was observed that explant and genotype exhibited significant differences in achieving the desirable results. Moreover, it was also found that shoot tip explant in onion is the best source for callus formation and plantlet regeneration. This protocol will pave way for future transformation experiments, to study the transient *gus* expression and for the development of transgenic plantlets.

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