GMR
The Original

EFFECT OF CALLUS INDUCTION MEDIA ON CALLUS FORMATION AND HAPLOID PLANTS REGENERATION IN ANTHER CULTURE OF BRINJAL (*SOLANUM MELONGENA L.*)

Vangapandu Thriveni1[*1], D. Sharma[2]

[1*]Assistant Professor, Department of Horticulture, MS Swaminathan School of Agriculture, Centurion University of Technology and Management, R-Sithapur, Paralakhemundi, Gajapathi district, Odisha, India, thriveni.vangapandu@cutm.ac.in,
[2] Principal Scientist, Department of Vegetable Science, College of Agriculture, Indira Gandhi Krishi Viswavidyala (IGKV),Raipur-492012, Chattisgarh, India, dsharma_hort@yahoo.co.uk

ABSTRACT:
The brinjal cross hybrid IGB-54 X KT was tested in seven different culture media C0, C1, C2, C3, C4, C5, N6 for haploid regeneration via antherculture, this study carried out at the IGKV, Raipur. The anthers were cultured in callus induction to optimize the frequency of embryonic calli production, size of the calli and day to callusing. Moreover, the androgenic calli that were unable to complete their growth in the culture medium studied were placed in regeneration treatments viz.T1, T2, T3, T4, T5 and T6. The highest number of calli(40.00) yielded with a frequency of 53.33 percent with maximum size of 7.70mm calli with moderate number of days 13.30 was obtained inIGB-54 x KT from the anthers cultured on C5 medium -MS medium containing 2mg/L 2,4-D+ 0.5mg/l kinetin, 30 g/L sucrose compared the Medium C4 and C2 .but in case of observation days to callusing earlier calli production was noticed in C4 9.98 days followed by C3(10.14 days), C2(11.76 days) and C5(13.30 days) slightly3.5 days late in initiation over C4 and 3.2 days over C3.Least number of calli(2.33), frequency(3.11 per cent, size 2.37 mm with a maximum of 54.25 days taken in N6 medium. Among the shoot regeneration treatments, the treatment T3 – 3 mg/l BAP +1mg/l Kinetin was found better for shoot regeneration with 79.48 % greening, highest number of calli initiated shoots (23.00) with shooting frequency of 74.20 percent, with moderate 5.25 average number of shoots/calli and shoot length of 4.00cm. However, it takes 122 days for regeneration in general haploid calli regenerates very slowly into shoot formation. In the same media embryo formation also noticed while calli regenerated with a frequency of (2calli) 2.56 per cent and produced 3 plants with plantlet frequency of 3.85 percent. Then rooted in MS +IBA 1mg/l rooting media and acclimatized in the hardening room. There about the cross IGB-54 × KT had a total of 159 plantlets produced, out of these 108 plants were hardened and 58 plants survived with a frequency of 53.70%. Almost all the plants derived from anther culture are 98 percent haploids.

KEYWORDS: Brinjal, Calli induction, shooting, haploid, antherculture.

INTRODUCTION

Brinjal is the most commonly cultivated crop of India. The term ‘eggplant’ applies to a large number of species of the genus Solanum (cultivated, semi wild, or wild) that bear fleshy berries (Daunay et al., 2001). However, Indio- Burma region is the primary centre of diversity because Solanum genus has vast diversification in diverse ecological conditions still, we can find most of the wild species of brinjal in anywhere in all over India. According to the data of the Food and Agriculture Organization, 42,944,212 t of eggplants were produced in an area of 1,676,893 ha in 2009 worldwide (http://faostat.fao.org). The highest eggplant production in the world is in China (25,912,524 t), which meets 60% of the world’s production. In brinjal has large flower crosses are easy to develop and considerable number of seeds can be obtained by conventional breeding; besides this some cross combinations are may advantageous in heterosis. A number of researcher’s studies shown that significant heterosis on yield, maturity, earlier uniform harvest and other traits in brinjal (Kaur et al 2001, Singh et al 2012.). The selection and development of homozygous lines is time consuming and difficult task to achieve. The researchers Germana,2006; Ferrie and Mollers, 2010 achieved about 98 % of homozygosity after 6 years of selfing. At present time, homozygous lines can generate by different haploidy methods.

The main method of obtaining haploid plants in eggplant is androgenesis. In vitro androgenesis in S. melongena has been commonly employed for the last 40 years (Dumas de Vaulx and Chambonnet, 1982; Rotino, 1996, 2005; Segui Simarro et al., 2011). It is possible to produce haploid (n) plants, which re-establish their normal ploidy level (2n) either spontaneously or after colchicine treatment. With this technique the time required to obtain homozygous material from the initial heterozygous material has been reduced (Daunay et al., 2001) Studies with anther culture have mostly been conducted for cultivated eggplant (S. melongena) with goal of obtaining double haploid parents for conventional breeding (Rotino, 1996). The double haploid plants have been successfully used in conventional breeding programs to obtain pure lines faster than selfed inbreds. Double haploid plants are homozygous at all loci, and this may help to study the genetic basis of quantitative traits by overcoming the problems associated with the envirobmental cariations. Raina and Iyer (1973) were first to report plant regeneration from anther culture in brinjal. They regenerated homozygous diploid (double haploids) plants through callus developed from anthers cultured at uninucleate pollen stage that were previously treated with colchicines. Haploid plantlets were also obtained from the Research group of Haploid breeding (1978) and Isouard et al. (1979) a year later. Dumas De Vaulx and Chambonnet (1982) did an extensive work to improve the development of

androgenic haploids. A combination of both auxin and cytokinin was essential during early stages of anther culture. Similarly, Rotino et al. (1987) showed that haploid plant regeneration was affected by genotype, temperature, culture conditions, hormones and anther stage.Sevral inuctive media and treatments used to produce DHs via anther culture in different brinjal F1 hybrids and cultivars under different experimental conditions(Sanguineti et al1990,Rotino,1996, Gemes Juhasz et al 2006,Alposoy and Senz,2007,Salas et al 2011).(Kothari et al., 2010 and Irikova et al., 2011 in pepper). Success in anther culture is predominantly dependent on the calli induction media and genotype of the anther donor material A combination of both auxin and cytokinin was essential during early stages of anther culture(Rotino 1987), in brinjal.

Keeping these points in view, the present investigation was carried out to investigate the responses from one hybrid on the production of androgenic derived calli cultured on MS medium supplemented with various growth regulators in different ratios and with standard pre-culture 3h duration and heat treatment of anthers at 25°C for 21 days. Similarly, However , the antherculture technique for haploid regeneration protocol in brinjal for economic higher yield production of the crosses IGB-54 ×KT to generate dh population for fixing the homogeneity in the further breeding programmes in brinjal in IGKV.

MATERIALS AND METHODS:

The cross hybrid IGB-54 × Kasi Taru (KT) was planted at Research Farm under ACRIP on vegetable crops, Department of Vegetable Science, IGKV, Raipur. Throughout the growing period, the normal cultivation practices were implemented to raise healthy plants. Plants were maintained free from insect-pests and diseases. To stimulate development of fresh flower buds, the young set fruits were removed periodically. During flowering period of plants, flower buds which were in the proper stage of anther development were collected and their anthers were cultured in different media.

Flower buds were collected at 9-10.5 mm size ie at petals reaches to sepal separation level with a anther size of 4.2-5.6 mm at late uninucleate stage to early binucleate stages of microspore development. Later the size and morphology of flower buds can be used as an indirect indication for determining the stage of microspore development. Anthers from flower buds of different stages were subjected to cytological examination by staining with 2 per cent acetocarmine dye after squashing and observed under microscope at 1000x magnification. These buds were washed with tap water and surface sterilized for one minute in 70 per cent ethanol followed by 30 sec-1 minute in a filter paper and washed with distilled water later kept in the refrigerator at 4-5°C chilling temperature for 1-3 hours duration. Then surface sterilized with HgCl2 for 1-3 min and rinsed 3 to 4 times with sterile distilled water to remove the traces of HgCl2. The anthers were aseptically removed from the buds carefully and were placed on Petri-plates containing C medium - MS media with different concentrations and combinations of hormones at 30 g/l sucrose Vize control, C0, C1, C2, C3, C4, C5 and N6 media. About 75-150 anthers from 30 plants were collected in the early morning between 7.00 am to 8.30am during winter and cultured for each treatment. The cultured anthers were given heat shock treatment by keeping the inoculated Petri-plates in incubator at 35oC under dark condition for 8 days as well as at 25°C for 3 weeks and then shifted the cultures to culture room at 25±2oC under photoperiod of 16 h light and 8 h dark. After three to four weeks, anthers were sub-cultured on same treatment for callus maintenance. Calli from anthers with size 5-7 mm were transferred to R medium Cultures were then incubated in culture room at 25±2°C temperature, under photoperiod of 16 hours light and 8 hours dark.

The number of days taken to show callo genesis from the date of inoculation, no of calli induced and Per cent response frequency of anthers to callus initiation in various treatments of C medium was recorded. The mean and standard errors were worked out from triplicate data obtained from various experiments. The per cent data transformed using angular transformation and analyzed following factorial Completely Randomized Design (CRD).The anther derived plantlets were hardened for 15-20 days in primary hardening room in small pots composed with garden soil, Cocopeat at 1:1 ratio and then shifted in big poly bags in the green house for 30-45 days.

Cytological studies were carried out using root-tip ceils of plants grown in rooting media, either MS or MS + IBA 0.05 mg/1 and 20 g/1 sucrose, with the exception of a small sample of plants grown from anther derived calli. Roots were pre-treated for 3--4 hours with 0.3 % colchicine at 18-19 °C in continuous shaking for 5-6 hours and then fixed in 3:1 absolute alcohol: glacial acetic acid for a minimum of 24 hours. The roots were hydrolysed in 1N HC1 at 60 °C for 7 minutes, rinsed with dis- tilled water and stained in 2 % acetocarmine with pinch of ferric chloride Squash preparations were made in aceto- carmine. Then the stain root tip placed on glass slide covered with coverslip pressed with the thumb for even distribution and tapped gently. Chromosome counts were established from a minimum of five well- spread cells and at least two different roots under microscope thoroughly screened for evidence of haploid and mixoploids.

They are different treatment combinations used for anther culture in brinjal for callus induction; shoot regeneration and rooting were in detailed enlisted in Table no1and 2

Table No.1: List of different culture media treatment combinations utilized for callus initiation/ embryo formation in anther culture

Type of media	Composition of media +hormone	Explant used	Reference
Callus induction	C0- MS+24-D 2m/l	Anther	De valux-1982, Prabhavati et al,2003
	C5-MS+24D 2mg/l + Kn 0.5mg/l		Modification Chen et al 2008
	C1 - MS+24-D 5mg/l		
	C2 - MS+ 24-D 5mg+Kn 5mg/l		Bassay et al.2012
	C3 - MS+BAP+NAA+GA3		

	C4 MS+NAA1mg/l +BAP1mg/l		Rotino 1966,Khatun et al 2006,
	N6+24-D2mg/l		Sujata et al.2014

Table 2.List of different Regeneration media treatment combinations utilized for proliferation of shoot and rooting in anther derived calli/embryos.

Type of media	Composition of media treatments	Explant used	References
Shoot regeneration media	T1- MS media with no harmone	Calli	Albha rivas et al 2017
	T2- BAP@ 2mg/l +kn 0.5mg/l	Calli	
	T3-BAP 3mg/l + kn 1mg/l	Calli	Dehkhan et al 2017
	T4 -BAP @4.4mg/l +kn 2.5mg/l	Calli	
	T5- BAP @0.5 mg /l + Kn 0.5 mg/l	Calli	
	T6- Kn @ 1mg/l	Calli	Dumas De valux, 1981
Root regeneration media	R5a-MS+ IBA 1mg/l	Shootlets	

RESULT AND DISCUSSION

Calli induction:

After pretreatment of flower buds, the buds were takeout and sterilized with 0.1 % HgCl2 for 3 minutes then wash five times with distilled water. The anther were dusted on petri plate containing solidified calli media supplemented with maltose 3%, agar 0.8 % and 2mg/l 2,4-D. Each petri plate was inoculated with excised anthers. Anthers were inoculated on each petri plate and incubated in 25±2˚C as well as at 35°C for 8 days darkness inside a BOD incubator for callus induction was performed. But at 35°C of 8 days darkness are unable to express because of little performance was observed. The effects of different callus induction media on different parameters of anther derived calli in Solanum melongena L. genotype IGB-54 x KT at 25°C heat shock treatment during incubation of culture in dark for 21 days on embryo or calli formation were examined. According to the results of the analysis of variance the media showed that the percentage of anther derived calli induction frequency was highly significant at 0.01 probability level and it ranges from 3.11 to 53.33 per cent. Significant differences were observed in the calli induction frequency (Table 3) due to media levels on cultured anthers in in vitro condition. However the highest percentage of anther derived calli induced frequency was obtained by the media C5- MS media supplemented with 2.0 mg/l 2,4-D+ 0.5 mg/l Kin (53.33%) and also by C2 -MS medium integration with 5mg/l Kn+5mg/l 2,4,-D (49.78%) and lowest calli responding frequency with N6 medium supplemented with 2,4-D @ 2ml/l (3.11%).

Table.3. Influence of different media on callus induction of the cross IGB-54 XKT at 25°C heat stress incubated for 21 days in darkness.

Treatment	No of anthers inoculated	No of anthers induced calli	Calli induction frequency (%)	Days taken for callusing	Calli diameter (mm)	colour of the callus	Nature of calli	Abundance of the callus
CA	150	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-	-	0
C0	120	17.56±0.56	23.62±0.66	17.33±1.33	4.47±0.23	Cw	2	1
C1	78	10.78±2.39	14.48±3.18	17.93±1.67	3.33±0.75	Trans parent Hw	1	2
C2	78	37.33±2.67	49.78±3.55	11.76±0.05	7.23±0.82	Pw	3	3
C3	75	15.89±3.46	19.29±4.30	10.14±0.25	4.03±0.80	Hw	2	1
C4	75	35.14±4.68	42.74±4.25	9.98±0.27	7.53±0.47	W	3	3
C5	73	40.00±2.08	53.33±2.78	13.30±0.61	7.70±0.56	Cw	3	3
N6	150	2.33±0.67	3.11±0.89	54.25±1.29	2.37±0.52	Pinkish white	2	1

C.D.		7.70	8.84	2.78	1.78			
SE(m)		2.55	2.92	0.92	0.58			
C.V.		3.60	4.13	1.30	22.26			

Among two different Media supplemented with different hormones the C5 media imposed with 2, 4-D @ 2mg/l +0.5 kin mg/l recorded the highest number of calli induction 40.00 fallowed by C2 and C4 and the least number of calli production had with N6 media integrated with 2, 4-D 2mg/L (2.33), however in case of control treatment i.e.MS media without hormone did not showed calli induction. Days taken for callusing had recorded significantly the minimum number of days required in C5 (9.98 days) on par with C3 (10.00 days) fallowed by C2 (11.25 days) and C5 (13.30 days) but in N6 takes maximum number of days for callus intiation (54.25 days), respectively,.As in case of size of calli (mm) ranging from2.37 to 7.70 mm, the maximum diameter of calli observed in anther cultured in C5 media incubated at 25°C incubated under dark condition(7.70mm) which is on par with C4media(7.53mm) fallowed by C2(7.23 mm) but lowest calli diameter acquired inN6 (2.37 mm).

It is evident from the results that the highest frequency was noticed in the C5 compared to other calli induction media. This might be due to media composition favouring the enhancement of the survival of anthers and provides pressure /strength to burst opens the pollen grains from the anther to induce calli or embryos. A calli induction media C5 exhibited the maximum number of calli (40.00), calli induction frequency (53.33%), optimum calli size (7.70mm) and takes moderated number of days for callus initiation (13.30 days). However in case of calli induction media C2 had better in no of calli production(37.33), frequency(49.78%), but small calli size(4.03 mm) and slightly 1.5 days(11.25days) earlier in initiation of calli over the C5 fallowed by C4. According to calli induction frequency preculture were ranked in descending order C5,C2,C4,CO, C3, C1 and N6 and their means 53.33 a , 49.78b , 42.74c , 23.62d , 19.29e , 14.48f,and3.11g , respectively. After three weeks of inoculation, abundance of callus was recorded and graded as 3 for plenty, 2 for moderate and 1 for poor. After three weeks of inoculation, nature of callus was recorded and graded as 3 for compact, 2 for friable and 1 for loses of its texture.

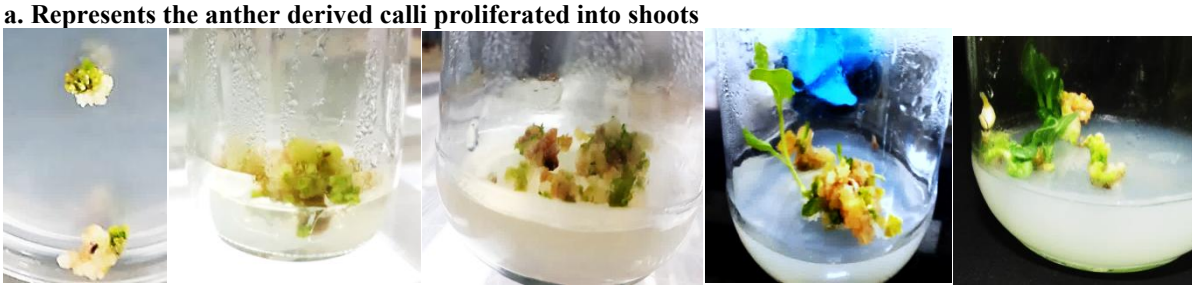
Shoot regeneration:

In responsive anthers the wall tissue gradually turned brown and after four weeks they burst to open due to pressure created excreted by the growing calli or embryos with probably from different origin (microspores or diploid cells of anther wall. They are three type of responses observed; some anthers had embryogenic calli, few of showed both embryos and calli, while some other produced embryos directly. For shoot regeneration, the calli obtained from the anthers of the IGB-54×KT were cultured on different regeneration media and the cultures were incubated in light. The produced calli multiplied and proliferated on same medium and then were transferred to shoot regeneration media supplemented with various concentrations of BAP, Kn, NAA and GA3 along with control vize T1,T2,T3,T4,T5 and T6.

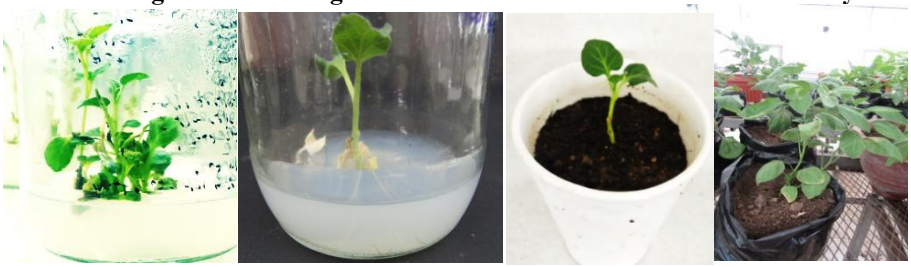
Plate 1.Calli/embryo formation and shoot regeneration of brinjal cross IGB-54 × KT via antherculture



a. Represents the anther derived calli proliferated into shoots



b. The androgenic calli on regeneration media shows few number of embryo formation



c. Rooting of shootlets and Hardening of androgenic calli derived plantlets

Plate 1.Calli/embryo formation and shoot regeneration of brinjal cross IGB-54 × KT via antherculture

Table: 4. Influence of different regeneration treatments on shoot regeneration of androgenic calli of the cross IGB-54 XKT in brinjal antherculture.

Treatment	No of calli inoculated	No of calli responded	% of calli responded	number of calli intiated shooting	shoot initiation response	Average number shoots/calli	Days to shooting	shoot length(cm)
T1	18.50	4.00	22.03	0.00	0.00	0.00	0.00	0.00
T2	24.50	16.50	67.59	6.50	40.63	1.00	56.00	4.20
T3	39.00	31.00	79.48	23.00	74.20	5.25	122.34	4.00
T4	38.00	29.00	76.53	14.00	48.28	6.00	173.50	3.15
T5	37.50	27.50	72.12	16.00	59.26	4.25	64.00	4.85
T6	12.00	10.00	83.34	4.00	33.34	4.13	68.50	3.14
C.D.		8.49	22.34	5.62	35.01	1.95	27.80	0.66
SE(m)		2.41	6.33	1.59	9.92	0.55	7.88	0.19
C.V.		12.05	13.38	21.30	32.09	22.71	15.62	10.52

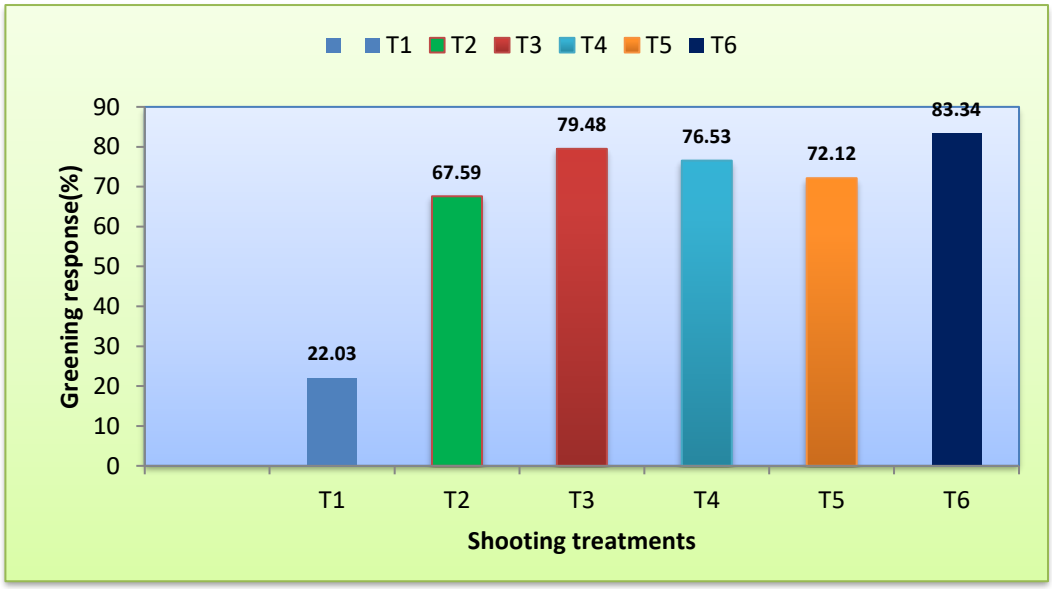


Fig.1. Influence of different regeneration treatments on greening (%) of the cross IGB-54 XKT in brinjal antherculture

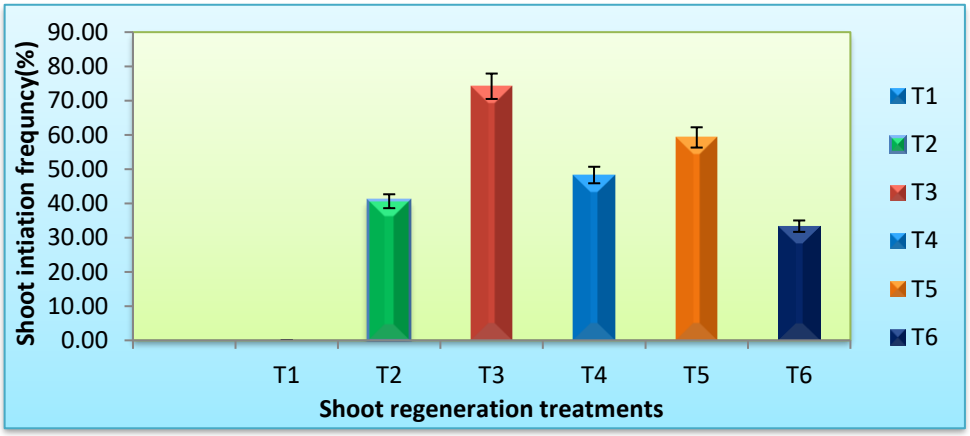


Fig.2. Influence of different regeneration treatments on greening (%) of the cross IGB-54 XKT in brinjal antherculture

The results of Table.4) indicated a significance differences between hormonal combinations at 1% probability level for the mean greening (%).The greening response in per cent showed (ranged from 22.03 -83.34%), however maximum greening had with T6 83.34% fallowed by T3(79..48 %) and T4 (76.53%) and minimum with T1 (22.03%) shown in Figure.no.1.Similarly the highest number of calli initiated shoots(23.00) with shooting frequency of 74.20 per cent (in fig.no.2) observed in T3, followed by T5 (16.00)59.26 per cent, while least obtained with T6-(4.00)33.34 per cent, respectively. But the maximum number of shoots regenerated per calli had in T4 (6.00) fallowed by T3 (5.25) and the maximum shoot length had in T5 fallowed by T2 and T3.However for the calli proliferated into shoots takes minimum number of days in T2 56.00 days fallowed by T5 (64.00 days) and T6 (68.50 days).But in T3 treatment it tooks 122 days for shoot regeneration.

Among the all the parameters showed the treatment T3 found better for shoot regeneration with 79.48 % greening, highest number of calli initiated shoots(23.00) with shooting frequency of 74.20 per cent, 5.25 average number of shoots/calli with moderate shoot length of 4.00cm.However it takes 122 days for regeneration in general haploid calli regenerates very slowly into shoot formation. In the same media embryo formation also noticed while calli regenerated on shoot regeneration medium with a frequency of (2calli) 2.56 per cent and produced 3 plants with plantlet frequency of 3.85 per cent.

The elongated shoots were put on MS media with GR free and full strength MS medium supplemented with IBA (1mg/l) for rooting as the roots were not induced spontaneously in the regeneration medium. After the development of roots, plantlets were hardened for two weeks in MS medium having half strength at 25 ± 2°C. Then the hardened plants were shifted to the plastic cups having mixture of soil, sand and vermiculite (1:1:1, v/v/v) and then these plants were shifted to pots after 2 weeks. Thus the protocol standardized in the present study may contribute to improvement of brinjal significantly.

Chromosome number and morphogenesis of androgenic calli derived plants.

Chromosome number in root tips of diploid brinjal plant and those of haploid plants from androgenic calli originated haploid plants.in brinjal about 56 plants were haploids and others were diploid or mixiploids. There are about The cross IGB-54 × KT had a total of 159 plantlets produced, out of this 108plants were hardened and 58 plants were survived with a frequency of 53.70%. Almost all the plants derived from anther culture are 98 percent are haploids. As we observed that plate no.3.the haploid plants are usually dwarf stout and short leaves, petioles, no flowering. Whereas diploids or DH populations are tall having fertile big size flowers and broad leaves. There was the morphological variability seen in diploid and haploids. The leaf of haploid brinjal hybrid IGB-54 X KT characteristically lacked the proximal half compared to diploid leaf and flowering was not noticed in haploids.



Plate no.3.Leaves and flowers of haploid and diploid brinjal hybrid IGB-54 × Kasi Taru

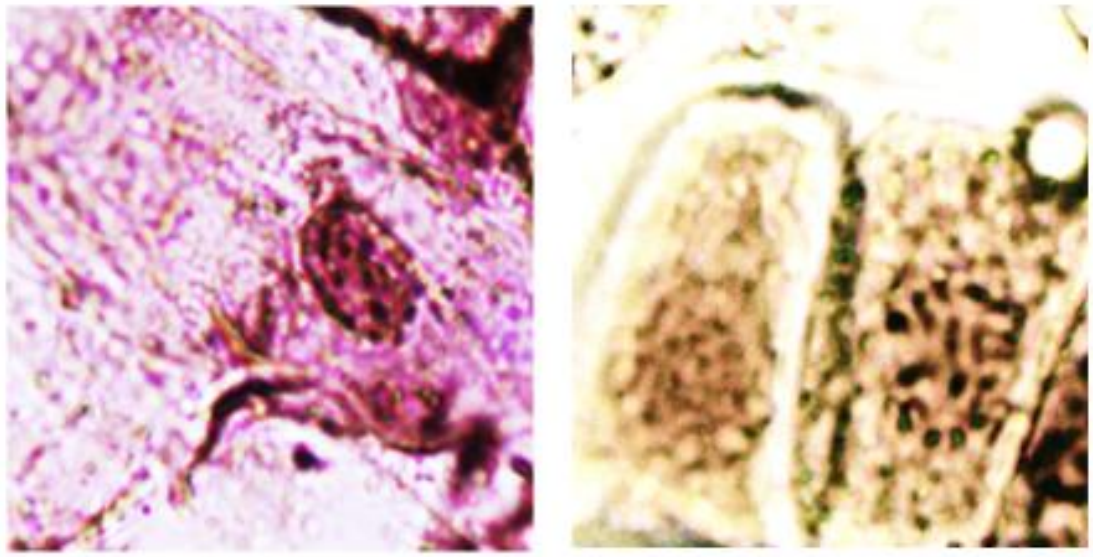


Plate no.4.Chromosomes identification in haploid and diploid plants of brinjal cross IGB-54 ×KT

DISCUSSION

The composition of media and phtyohormone concentration in media are key factors in determining plant production from anthes and subsequent development of the plant. Liljana et al 2007 reported in incubating treatment in heat conditions (at 25°C) in darkness for 7 days, and then transferring the explants to light conditions (12-h photoperiod at 25 °C) for 4 weeks, on MS anthers produced callus we also get similar results in IGB-54 X KT the flower buds were pre-treated at5°C for 3 h and incubated at 25°C heat stress for 21 days in dark condition induced androgenic calli. Results on effect of calli induction media supplemented with three concentrations of individual plant growth regulators like BAP, kinetin, NAA, GA3 and 2, 4-D at 0.2mg/l at heat treatment at 25°C for 21 days on androgenic response of brinjal hybrid IGB-54 × KT was presented in Table 4. Anther response is enhanced with the suitable balance of auxins and cytokinins in the culture medium. In the present experiment, the results showed the need for growth regulators i.e. both auxins and cytokinins to achieve androgenesis from anthers. Callus induction frequency also depends on type of auxins and cytokinins used (BAP, kinetin, 2, 4-D, NAA and GA3). The growing medium supplemented with2 mg/l 2, 4-D and 0.5 mg/l kin was found superior for callus induction from cultured anthers followed C2-2,4-D 5mg/l +5mg/l kinetin). Beneficial effect of auxin and cytokinins added to the culture media on haploid callus and embryo induction was reported by Nowaczyk et al., (2006); Supena and Custers (2011); Lantos et al., (2012); Luitel and Kang (2013) and Olszewska et al., (2014) in pepper. In the hybridIGB-54 XKT, the percentage of callusing anthers was significantly higher on medium supplemented with kinetin 0.5 at mg/l along with 2, 4-D at 2 mg/l (Plate 2b&cCallus induction response under BAP was the poor as compared to other cytokinins tested in brinjal hybrid IGB-54 XKT.

Its evidenced that Among different calli induction media the maximum calli frequency observed in the C media having both auxins and cytokinins C5-MS media augmented with 2mg/l2,4-D and 0.5 mg/l kinetin and C2-5mg/l 2,4-D and 5mg/l kinetin supported with the results of Bassay et al 2013. Success in anther culture is predominantly dependent on the calli induction media and genotype of the anther donor material A combination of both auxin and cytokinin was essential during early stages of anther culture(Rotino 1987), in brinjal.(Kothari et al., 2010 and Irikova et al., 2011 in pepper). Sevral inuctive media and treatments used to produce DHs via anther culture in different brinjal F1 hybrids and cultivars under different experimental conditions(Sanguineti et al1990,Rotino,1996, Gemes Juhasz et al 2006,Alposoy and Senz,2007,Salas et al 2011).

Cytokinin and auxin are the commonly known plant growth regulators being widely used in explant regeneration as different combination ratios of these two would bring significant difference in regeneration processes. Cytokinins are commonly used in plant tissue culture to initiate cell growth and also to induce the formation of shoots whereby there are actively take part in various plant physiological processes (Davies 1995). This includes shoot multiplication (Kaviani et al. 2013; Bhat et al. 2013; Abu-Romman et al. 2015), tuberous root production (Deshwal & Trivedi 2011), callus induction (Borjian & Arak 2013), leaf senescence (Riefler et al. 2006) and shoot apical meristem activity regulators (Tucker & Laux 2007). Cytokinin such as kinetin is known to initiate cell division in the presence of auxin and is widely used together with auxins for callus formation or to induce shoots where levels of auxin low is available. Cytokinin like 6-Benzylaminopurine (BAP) or benzyl adenine on the other hand is used for growth acceleration.

Previous regeneration studies in Solanum melongena L. reported the use of kinetin in combination with other plant growth regulators to induce shoots. Sarker et al. (2006) used a combination of kinetin and BAP to induce a high number of shoots, ranging from 0.75 to 4.4 shoots per explant in the cultivar Singhnath and Kazla. Jamil et al. (2013) reported that kinetin is necessary to be included together with other plant growth regulators in inducing shoots for eggplant cultivar NS-797. Robinson and Saranya (2013) also reported a high number of shoot induction (5.9 ± 2.5 shoots per explant) with a percentage of 90% of shoot formation was achieved by supplementing 1.5 mg/L BAP in the culture media indicated the efficiency of BAP in shoot induction for a different cultivar

There were no shoot and callus formation observed in full-strength MS basal medium without any plant growth regulators whereby only the formation of roots was noted in IGB-54 X KT. But previous researchers reported the rooting has been successfully induced on MS basal medium without plant growth regulators in other eggplant cultivars such as cv. Pusa Purple Long, cv. Black Jack and cv. Loda (Chen et al. 1995; Rahman et al. 2006; Pratap et al. 2011) and suggested the MS basal medium without plant growth regulators can be used for root regeneration. Then shoots were transferred to MS supplemented with 1mg/l IBA. These results also indicated that this cultivar of Solanum melongena L. does require auxins for root production in haploid regeneration though anther culture because haploid shootlets slow growth it takes more number of days for root regeneration. Therefore IBA is the most commonly used rooting harmone for root regeneration in invitro rhizogensis of shootlets.

The parental cross hybrid IGB-54 ×KT androgenic calli regenerants about 159 were produced and almost 108 plants were identified as haploids and acclimatized with a survival frequency of 53.70 per cent. The plants which were identified to be haploid were taken and further used for double with Invivo application of colchicine given axillary bud at 4-6 leaf stage androgenic calli derived regenerants for fixing the homogeneity into DHs were grown in Invivo condition in the growth chamber for a month. Then the ploidy levels were rechecked by the method, Chromosome count. Almost all the plants derived from anther culture are 98 percent are haploids. It was not clear whether they were derived from the pollen grains or diploid cells of anther wall. As we observed the haploid plants are usually dwarf stout and short leaves, petioles, no flowering. Whereas diploids or DH populations are tall having fertile big size flowers and broad leaves. There was the morphological variability seen in diploid and haploids.

REFERENCE

1. SaAlba Rivas-Sendra, Manuel Campos-Vega, Antonio Calabuig-Serna, Jose M. and SeguiSimarro (2017) Development and characterization of an eggplant (Solanum melongena L.) doubled haploid population and a doubled haploid line with high androgenic response Euphytica, 213:89.
2. Anonymous. 2018. Area and production statistics of vegetables‟ in India, Indian Horticulture data base.
3. Chen Q, Jelenkovic G, Chin C K, Billings S, Eherhardt J, Goffreda J C and Day P. (1995). Transfer and transcriptional expression of Coleopteran CryIIIB endotoxin gene of Bacillus thuringiensis in eggplant. Journal of the American Society for Horticultural Science 120(6): 921–927
4. Chée PP and Cantliffe DJ (1990). Selective enhancement of Ipomoea batatas poir embryogenesis and non-embryogenic callus growth and production of embryos in liquid culture. Plant Cell Tissue Organ Cult. 15: 795-797.
5. FAO. 2016-17. Statistics. http://faostat.fao.org
6. Daunay M. C., Lester R. N. and Ano, G. 2001. Eggplant. In: Charrier A, Jacquot M, Hamon S and Nicolas D, editors. Tropical Plant Breeding. Enfield, NH, USA: Science Publishers Inc., pp. 199– 222.
7. Dumas de Vaulx, R., Chambonnet, D. and Pochard, E. 1981. In vitro anther culture in red pepper (Capsicum annuum L.): improvement of the rate of plant production in different genotypes by treatments at 35oC. Agronomie, 1: 859– 864.
8. Dumas de Vaulx R., Chambonnet D.1982. Culture in vitro d‟anthèresd‟aubergine (Solanum melongena L.): stimulation de la production de plantes au moyen de traitements á +35 °C associesa de faiblesteneurs en substances de croissance. Agronomie 2: 983–988 (in French).
9. Ferrie AMR, Mo¨llers C (2010) Haploids and doubled haploids in Brassica spp. for genetic and genomic research. Plant Cell, Tissue Organ Cult 104:375–386.
10. Germana` MA (2006) Doubled haploid production in fruit crops. Plant Cell, Tissue Organ Cult 86:131–146
11. Gemes Juhasz A., VenczelG., Sagi Z.S., Gajdos L.,Kristof Z., Vagi P.,and Zatyko L.(2006)Production of double haploid breeding lines in case of paprika, spice paprika, eggplant, cucumber, zucchini and onion. In:Acta Horticulturae. International Society for Horticultural Science(ISHS)Leuven Belgium, 845-854.
12. Gulshan,T,M., Varghese, Sharma,D,R.1981 studies on anther cultures of tomato Lycopersicon esculentum Mill. Biologia Plantarum, Prague,v.23,n.6,p.414-420.
13. Irikova, T., Grozeva, S. and Rodeva, V. 2011. Anther culture in pepper (Capsicum annuum L.) in vitro. Acta Physiologiae Plantarum, 33: 1559-1570.
14. Isouard G, Raquin C, Demarly Y (1979) Obtention de plantes haploides et diploides par culture in vitro d'anthe`res da´ubergine (Solanum melongena L.). C R Acad Sci Paris 288:987–989
15. Jamil M D, Parvaiz M, Tufail M, Arshad J, Hussain S and Imtiaz S. (2013). Callogenesis, regeneration of shoot and root of brinjal (Solanum melongena L.). World Applied Sciences Journal 26: 1039–1045.
16. Kaur.J,Patel, J.A., PatelM.J., Bhanvadia A.S., and Acharya.R.R.(2001).Heterosis for fruit yield and its components in brinjal (Solanum melongena L.).Capsicum Eggplant Newsl, 20.102-105.
17. Khatun F, Meah MB, Nasiruddin KM (2006). Regeneration of eggplant through anther culture. Pakistan J Biol Sci 1: 48–53.
18. Kothari, S.L., Joshi, A., Kachhwaha, S. and Ochoa, A.N. 2010. Chilli peppers—a review on tissue culture and transgenesis. Biotechnol. Adv., 28: 35-48.
19. Luitel, B.P. and Kang, W.H. 2013. In vitro androgenic response of minipaprika (Capsicum annuum L.) genotypes in different culture media. Hort. Environ. Biotechnol., 54(2): 162-171.
20. Lantos, C., Juhasz, A.G., Vagi, P., Mihaly, R., Kristof, Z, and Pauk, J. 2012. Androgenesis induction in microspore culture of sweet pepper (Capsicum annuum L). Plant Biotechnol. Reports, 6: 123-132.
21. Masoud Emrani Dehkehan, Ahmad Moieni and Zahra Movahedi (2017) Effects of BAP, Kin and NH4NO3 concentration on the eggplant anther culture (Solanum melongena L.) Iranian Journal of Field Crop Science vol 48 (3) :877-888.
22. Nowaczyk, P. and Kisiala, A. 2006. Effect of selected factors on the effectiveness of Capsicum annuum L. anther culture. J. Applied Genetics, 47(2): 113-117.
23. Olszewska, D., Kisiala, A., Niklas, N.A. and Nowaczyk, P. 2014. Study of in vitro anther culture in selected genotypes of genus Capsicum. Turkish J. Biol., 38: 118-124.
24. Pablo Salas, Jaime Prohens, Jose M. and Segui Simarro. 2011. Evaluation of androgenic competence through anther culture in common eggplant and related species, Euphytica 182:261–274.
25. Pratap D, Kumar S, Raj S K and Sharma A K. (2011). Agrobacterium-mediated transformation of eggplant (Solanum melongena L.) using cotyledon explants and coat protein gene of cucumber mosaic virus. Indian Journal of Biotechnology 10(1): 19–24.
26. Rahman M, Asaduzzaman M, Nahar N and Bari M A. (2006). Efficient plant regeneration from cotyledon and midrib derived callus in eggplant (Solanum melongena L.). Journal of Biosciences 14: 31–38.
27. Robinson J P and Saranya S. (2013). An improved method for the In Vitro propagation of Solanum melongena L. International Journal of Current Microbiology and Applied Sciences 2(6): 299–306.
28. Raina SK, Iyer RD (1973). Differentiation of diploid plants from pollen callus in anther cultures of Solanum melongena L. Z Pflanzenzucht 70: 275–280.
29. Rotino GL (1996). Haploidy in eggplant. In: Touraeu, A, Forster BP, Jain SM, editors. Advances in Haploid Production in Higher Plants. Berlin: Springer–Verlag, pp. 199–208.
30. Rotino GL, Falavigna A, Restaino F (1987). Production of antherderived plantlets of eggplant. Capsicum Newsletter 6: 89–90.

31. Rotino GL, Sihachakr D, Rizza F, Valé G, Tacconi MG, Alberti P, Mennella G, Sabatini E, Toppino L, D'Alessandro A (2005). Current status in production and utilization of dihaploids from somatic hybrids between eggplant (Solanum melongena L.) and its wild relatives. Acta Physiol Plant 27: 723–733.
32. Sarker R H, Yesmin S and Hoque M I. (2006). Multiple shoot formation in eggplant (Solanum melongena L.). Plant Tissue Culture and Biotechnology 16(1): 53–61.
33. SanguinetiM.C., Tuberosa R., and Conti S.(1990). Field evaluation of androgenic lines of eggplant. Acta Horticulturae, 280:177-182.
34. Segui-SimarroJ.M., Corral-Martinez P.,Parra-Vega V.,and Gonzalez-Garcia B.(2011). Androgensis in recalcitrant solanaceous crops.Plant Cell Reports, 30:765-780.
35. Sevinç BAŞAY, Şeküre Şebnem Ellialtioğlu(2013) Effect of genotypical factors on the effectiveness of anther culture in eggplant (Solanum melongena L.). Turk J Biol (2013) 37: 499-505.
36. Singh K.,Sindhu A.S., and Kumar A.(2012). Heterosis for fruit yield and its components in brinjal (Solanum melongena L.). Journal of Horticultural Science,7(2):142-144.
37. S V Bhat, A S Jadhav, B D Pawar, A A Kale, V P Chimote and S V Pawar (2013) In vitro shoot organogenesis and plantlet regeneration in brinjal (Solanum melongena L), The Bioscan,8(3): 821-824.
38. Supena, E.D.J. and Custers, J.B.M. 2011. Refinement of shed-microspore culture protocol to increase normal embryos production in hot pepper (Capsicum annuum L). Scientia Horticulturae, 130:769-774.