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Invitro Haploid Plantlet Regeneration through Anther Culture in Locally Adapted Cultivar of Indian Mustard (*Brassica juncea* L. Czern and Coss)

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A B S T R A C T

Anthers from four genotype of Indian mustard, namely Local Yella (CAULC-1), JD-6, Kranti and NDRE-22) were cultured *in vitro* to observe their androgenic responses. Different concentrations and combinations of growth regulators were supplemented in B_5 and MS medium. The range of callus induction was between 16.11 and 66.39%. The maximum rate of callus induction in terms of percentage (%) was observed in MS + 3 ml/l 2, 4-D + 1ml/l BAP (66.39). Among the Genotypes CAULC-1 (Local Yella) showed the best performance for days to callus initiation and per cent of callus induction while NDRE-22 was the poorest. Maximum per cent of shoot initiation at 30 and 45 DAI (55.55% and 59.44% respectively) was observed in MS + 4 ml/l BAP + 1 ml/l NAA. Local Yella (CAULC-1) recorded the best for shoot regeneration followed by Kranti. The media composition MS + 1 ml/l NAA + 0.5 ml/l BAP showed the minimum days (12.97) and highest per cent of root initiation (45.83%) and also maximum length of regenerated root (3.42 cm) respectively. Local Yella was found to be the best genotype which took minimum time (12.44 days). Regeneration of haploid plants was confirmed by cytological examinations of the root tips of the plantlets from the callus of anther culture. The present study can be concluded that Local Yella (CAULC-1) showed better androgenic response than other genotypes under study.

Introduction

Rapeseed - mustard is one of the most important edible oil crops which accounts for 22.6% of the total oil produced and the production ranks second among all the oil crops in India (Anonymous, 2013). Oil of Indian mustard (*Brassica juncea*) is consumed in large quantity and it covers 85-90% of the total area under cultivation of rapeseed-mustard in India (Anonymous, 2013). In Manipur, the crop is grown just after the harvest of rice but productivity is far below the national level resulting into low production. Among the locally grown genotypes, Local Yella (CAULC-1) is one of them which is without any genetic intervention. Against this, background

attempts were made to provide several embryos and plantlets for locally adapted genotypes and early maturing mustard varieties. For a better breeding programme, it should have broad genetic base. The existing genetic base we have is not enough to meet the challenge.

To create variability and for their utilization, it is necessary to go for hybridization and selection of desirable types from the succeeding generation. Traditionally, homozygosity of the cross products is usually achieved by self-fertilization which is a time consuming process (Morrison and Evans, 1988).

To this regard, haploid production through anther culture along with double haploid (DH) production technique can play a significant role to reach the homozygosity in a shorter period of time. *In vitro* haploid production technique through anther culture provides rapid development of homozygous lines and has potential for more application as a versatile genetic manipulation tool for evaluation of desirable genotypes. Anther culture and subsequent plant regeneration offer an alternative and efficient technique to conventional breeding method and enable production of several plants from single anther.

It has also attracted considerable attention as a supplementary tool for the production of inbred lines and for obtaining hybrid cultivars. But this *in vitro* regeneration of *Brassica* through anther culture technique is used quite limitedly in our country.

So, there is indispensable need for studying the anther culture technique for improvement of *Brassica*. Therefore, the present investigation was undertaken to observe the androgenic responses of Indian mustard genotypes to anther culture.

Materials and Methods

Materials

Local genotype of Indian mustard, Local Yella (CAULC-1) along with JD-6, Kranti, and NDRE-22 were used for the present study and the seed materials collected from Department of Plant Breeding and Genetics, College of Agriculture, Central Agricultural University, Imphal, Manipur. Different media compositions were used to study the regeneration potentiality.

Media used for anther culture

B_5 and MS media supplemented with 1.0, 2.0 and 3.0 ml/l 2,4-D along with same concentration of 1.0 ml/l BAP were used for callus induction. Likewise, both the media B_5 and MS supplemented with 4.0, 5.0, 6.0 ml/l BAP along with the constant concentration of 1.0 ml/l NAA were used for shoot initiation, while for root initiation, both the media supplemented with 1.0, 2.0 and 3.0 ml/l NAA with constant addition of 0.5 ml/l BAP were used.

Anther culture technique

The unopened flower buds of all the four genotypes were collected when the microspores were at early to late uninucleate stage before 10 a.m. The collected flower buds were wrapped in the aluminium foil and kept in the refrigerator at 4°C for 24 hours. After the cold treatment, they were surface sterilised under aseptic conditions in a laminar air flow chamber. They were rinsed in 0.1 per cent $HgCl_2$ for 1 minute with intermittent shaking followed by three washings with sterile distilled water. The flower buds were opened with the help of sterile forceps and the six anthers were clipped off from each floret without damaging the anther wall and inoculated on sterile test tubes and incubated

at 25°±1°C temperature in complete dark condition for callus induction and checked to record the response. Thirty anthers of each genotype were inoculated into each treatment. Six to seven weeks after inoculation of anthers, the regenerated calli attained convenient size. Then they were removed aseptically from the existing medium to a sterilised petriplate and cut into pieces and placed them into the test tubes with shoot initiation media and incubated in 25°C±1°C with 16 hours photoperiod. Repeated sub-cultures were done at an interval of 15 days. When the sub-cultured calli were proliferated and differentiated into shoots and grew up to 2-3 cm in length, they were rescued aseptically and were separated from each other and again cultured on test tubes with freshly prepared root induction medium to induce root. Day to day observation was carried out to note the response of the growing plantlets.

Cytological analysis

The ploidy level of the plant regenerated from anther culture was investigated by standard acetocarmine root tip squashing technique (Darlington and Lacour, 1976). The 0.5 cm long root tips were fixed in 1:3 (glacial: ethanol) by volume for 24 hrs. The fixed root tips were washed thoroughly in running water to wash out the fixative. Then, the washed root tips were preserved in 70% ethanol for used. A root tip was taken in a clean slide and milky meristematic tissue was retained and all other parts were discarded and the tissue was allowed to stain for 20 minutes in 20 % acetocarmine stain. The stained tissue was softened with 0.1 N HCl for 50 seconds and warmed over spirit lamp and the tissue was squashed with a needle and a cover slip was placed over it. Then a filter paper was placed over the coverslip and gentle pressure was applied for further flattening and spreading of the chromosome and observed under Olympus phase contrast microscope at 100X.

Parameters recorded

In callus induction, the parameters recorded were the days to callus induction, per cent of callus induction at 10, 20 and 30 days after inoculation (DAI). For shoot and root initiation, the parameters recorded were days to shoot and root initiation, per cent of shoot and root initiation at 15, 30 and 45 DAI and length of regenerated shoot and root at 30,45 and 60 DAI respectively.

Statistical analysis

The data on various parameters of callus induction, shoot and root initiation were analysed in Factorial Randomised Block Design (FRBD) to obtain the effect of various treatments, genotypes and their interaction. Wherever necessary, data pertaining to different parameters were subjected to angular and square root transformation.

Results and Discussion

Callus induction

Callus induction performances of the genotypes, treatment compositions and genotype × interaction were evaluated and the results are presented in Tables 1, 2 and 3 respectively. The two factors *viz*., genotypes and treatment compositions were found to be statistically significant for the parameter of days to callus induction and per cent of callus induction after 30 days of inoculation. Out of the four genotypes, days required for callus induction was minimum in Local Yella (25.61) followed by JD-6 (27.01). On the other hand, genotype NDRE-22 took more number of days for callus induction (28.48). The differential response of different genotypes for days to callus induction were also reported by Alam *et al.,* (2009), Khan *et al.,* (2009) and Sayem *et al.,* (2010). In respect of the per cent of callus induction, Local Yella

produced maximum calli (53.67%) which was significantly higher than all other genotypes. It was followed by JD-6 and Kranti which gave callus induction of 51.59% and 49.08% respectively and the minimum was observed in NDRE-22 (37.96%). Among the six treatment compositions of the media containing different concentrations of the phytohormones, B_5 + 3 ml/l 2,4-D + 1 ml/l BAP required minimum time (25.86 days) for callus induction which was found to be statistically at par with MS + 3 ml/l 2,4-D + 1 ml/l BAP (26.20 days). Both MS + 1 ml/l BAP (28.00 days) and B_5 + 1 ml/l 2,4-D + 1 ml/l BAP (28.58 days) took maximum time for callus induction which were found to be statistically at par with each other. For the per cent of callus induction, MS + 3 ml/l 2,4-D + 1 ml/l BAP was the most responsive medium with an average of 66.39% callus induction followed by B_5 + 3 ml/l 2,4-D + 1 ml/l BAP (63.06%). As the concentration of 2,4-D was decreased, there was a decrease in callus induction.

The minimum percentage of callus (16.11%) was recorded in MS + 1 ml/l 2, 4-D + 1 ml/l BAP. From the results, it was observed that the performance of media with these concentrations of 2,4-D were significantly different suggesting that both days to callus induction and per cent of callus induction were greatly influenced by the concentrations of 2,4-D used. The result is in consonance with the results of Narasimhulu and Chopra (1987), Ockenden and Mc Cleriaghan (1993) and Sayem *et al.,* (2010). The genotype × treatment interaction was found to be significant for days to callus induction. Early callusing was found in the interaction of Local Yella with B_5 + 3 ml/l 2,4-D + 1 ml/l BAP (23.33 days) which was found to be statistically at par with MS + 3 ml/l 2,4-D + 1 ml/l BAP (23.78 days). Similar results were reported by Khan *et al.,* (2009) and Sayem *et al.,* (2010). But for per cent of callus induction, the interaction was found to be non-significant suggesting that all the four genotypes performed and responded equally in all the treatments under study which was found in agreement with the findings of Alam *et al.,* (2009).

Shoot initiation

Significant variations were observed among the genotypes and treatments for days to shoot initiation. Among the genotypes, Local Yella started shoot initiation early (24.59 days) as compared to other genotypes JD-6 (26.63 days), Kranti (27.41 days) and NDRE-22 (28.85 days) as shown in Table 4. The results also indicated that the media containing B_5 + 4 ml/l BAP + 1 ml/l NAA required minimum time for shoot initiation (24.47 days). On the other hand, both MS + 6 ml/l BAP + 1 ml/l NAA (28.70 days) and B_5 + 6 ml/l BAP + 1 ml/l NAA (28.36 days) took maximum time for shoot initiation (Table 5). Similar results were also revealed by Khan *et al.,* (2009) and Sayem *et al.,* (2010); who, found significant variations among the media with different hormonal concentrations. The genotype × treatment interaction was found to be significant for days to shoot initiation. Local Yella × B_5 + 4 ml/l BAP + 1 ml/l NAA took the lowest time (22.78 days), whereas, NDRE-22 × B_5 + 6 ml/l BAP + 1 ml/l NAA took the highest time for shoot initiation (30.11 days) which is in concordance with the findings of Sayem *et al.,* 2010 that revealed significant differences among the interaction (Table 6). The per cent of shoot initiation was significant at 30 and 45 DAI for genotypes, treatments and their interaction. For genotypes, the regeneration performances were found better in Local Yella (51.48%) followed by Kranti (48.15%) at 30 DAI. At 45 DAI, it was found better in Kranti (54.82%) followed by Local Yella (54.07%). Different concentrations of BAP showed significant variations for per cent of shoot initiation.

Table.1 Response of genotypes on callus induction

Genotypes	Days to callus induction	Per cent (%) callus induction after		
		10 DAI	20 DAI	30 DAI
Local Yella	25.61	0.00	0.00	53.67 (47.73)
Kranti	27.96	0.00	0.00	49.08 (44.20)
JD-6	27.01	0.00	0.00	51.59 (44.83)
NDRE-22	28.48	0.00	0.00	37.96 (37.57)
S.Ed. (±)	0.25	-	-	1.06
C.D. (0.05)	0.50	-	-	2.14

Figures in parenthesis are angular transformed values.

Table.2 Performance of media with different concentrations of phytohormones for callus induction

Treatment	Treatment composition	Days to callus induction	Per cent (%) of callus induction after		
			10 DAI	20 DAI	30 DAI
T_1	MS + 1 ml/l 2,4-D + 1 ml/l BAP	28.00	0.00	0.00	16.11 (23.53)
T_2	MS + 2 ml/l 2,4-D + 1 ml/l BAP	27.58	0.00	0.00	60.56 (51.70)
T_3	MS + 3 ml/l 2,4-D + 1 ml/l BAP	26.20	0.00	0.00	66.39 (54.66)
T_4	B_5 + 1 ml/l 2,4-D + 1 ml/l BAP	28.58	0.00	0.00	25.10 (28.83)
T_5	B_5 + 2 ml/l 2,4-D + 1 ml/l BAP	27.39	0.00	0.00	56.34 (49.55)
T_6	B_5 + 3 ml/l 2,4-D + 1 ml/l BAP	25.86	0.00	0.00	63.06 (53.21)
	S.Ed. (±)	0.30	-	-	1.30
	C.D. (0.05)	0.61	-	-	2.62

Table.3 Effect of genotype × treatment on callus induction

Treatment	Treatment composition	Genotypes	Days to callus induction	Per cent (%) callus induction after 30 DAI
T_1	MS + 1 ml/l 2,4-D + 1 ml/l BAP	Local Yella	26.89	18.89 (25.75)
		Kranti		
		JD-6	28.56	17.78 (24.92)
		NDRE-22	27.78	16.67 (24.03)
			28.78	11.11 (19.42)
T_2	MS + 2 ml/l 2,4-D + 1 ml/l BAP	Local Yella	26.11	66.67 (56.88)
		Kranti		
		JD-6	28.22	62.22 (52.10)
		NDRE-22	27.56	65.56 (54.10)
			28.44	47.78 (43.72)
T_3	MS + 3 ml/l 2,4-D + 1 ml/l BAP	Local Yella	23.78	72.22 (58.26)
		Kranti		
		JD-6	28.22	66.67 (54.75)
		NDRE-22	25.89	68.89 (56.14)
			27.89	57.78 (49.49)
T_4	B_5 + 1 ml/l 2,4-D + 1 ml/l BAP	Local Yella	28.11	28.89 (32.45)
		Kranti		
		JD-6	28.78	26.67 (31.06)
		NDRE-22	28.22	31.77 (27.78)
			29.22	16.67 (24.03)
T_5	B_5 + 2 ml/l 2,4-D + 1 ml/l BAP	Local Yella	25.44	64.24 (55.10)
		Kranti	27.89	55.56 (48.22)
		JD-6	27.45	58.89 (51.45)
		NDRE-22	28.78	46.67 (43.09)
T_6	B_5 + 3 ml/l 2,4-D + 1 ml/l BAP	Local Yella	23.33	71.11 (57.59)
		Kranti	27.11	65.56 (54.16)
		JD-6	25.22	67.78 (55.46)
		NDRE-22	27.78	47.78 (45.64)
S.Ed. (±)			0.61	2.60
C.D. (0.05)			1.22	NS

Table.4 Response of genotypes on shoot initiation

Genotypes	Days to shoot initiation	Per cent (%) shoot initiation after			Length (cm) of regenerated shoot after		
		15 DAI	30 DAI	45 DAI	30 DAI	45 DAI	60 DAI
Local Yella	24.59	0.00	51.48 (45.86)	54.07 (47.38)	0.55 (1.02)	1.30 (1.34)	2.55 (1.83)
Kranti	27.41	0.00	48.15 (44.58)	54.82 (47.83)	0.45 (0.97)	1.09 (1.25)	2.40 (1.70)
JD-6	26.63	0.00	41.11(39.61)	41.85 (40.04)	0.43 (0.96)	0.99 (1.22)	2.34 (1.69)
NDRE-22	28.85	0.00	30.48 (31.28)	33.48 (34.21)	0.32 (0.90)	0.86 (1.16)	2.09 (1.61)
S.Ed. (±)	0.27	-	1.92	1.43	0.02	0.02	0.06
C.D. (0.05)	0.55	-	3.86	2.88	0.04	0.04	0.13

Figures in parentheses are transformed values.

Table.5 Performance of media with different concentrations of phytohormones for shoot initiation

Treatment	Treatment composition	Days to shoot initiation	Per cent (%) shoot initiation after			Length (cm) of regenerated shoot after		
			15 DAI	30 DAI	45 DAI	30 DAI	45 DAI	60 DAI
T_1	MS + 4 ml/l BAP + 1 ml/l NAA	25.61	0.00	55.55 (48.25)	59.44 (50.54)	0.53 (1.01)	1.22 (1.29)	2.48 (1.73)
T_2	MS + 5 ml/l BAP + 1 ml/l NAA	27.25	0.00	46.86 (43.39)	50.56 (45.33)	0.39 (0.94)	1.01 (1.23)	2.29 (1.67)
T_3	MS + 6 ml/l BAP + 1 ml/l NAA	28.70	0.00	32.78 (33.12)	34.10 (35.48)	0.27 (0.87)	0.80 (1.14)	2.13 (1.62)
T_4	B_5 + 4 ml/l BAP + 1 ml/l NAA	24.47	0.00	50.00 (45.97)	56.11 (48.56)	0.66 (1.08)	1.33 (1.35)	2.58 (1.89)
T_5	B_5 + 5 ml/l BAP + 1 ml/l NAA	26.83	0.00	44.42 (41.88)	36.67 (42.52)	0.46 (0.98)	1.10 (1.26)	2.46 (1.70)
T_6	B_5 + 6 ml/l BAP + 1 ml/l NAA	28.36	0.00	27.22 (29.40)	29.45 (31.76)	0.33 (0.91)	0.90 (1.18)	2.18 (1.64)
S.Ed. (±)		0.34	-	2.35	1.75	0.02	0.03	0.08
C.D. (0.05)		0.68	-	4.73	3.53	0.05	0.05	0.15

Figures in parenthesis are transformed values.

Table.6 Effect of genotype × treatment on shoot initiation

Treatment	Genotypes	Days to shoot initiation	Per cent (%) shoot initiation after		Length of regenerated shoot after		
			30 DAI	45 DAI	30 DAI	45 DAI	60 DAI
T_1	Local Yella	23.56	60.00 (50.81)	66.67 (54.81)	0.63 (1.06)	1.45 (1.40)	2.70 (1.79)
	Kranti	25.22	57.78 (49.64)	64.44 (53.52)	0.55 (1.03)	1.25 (1.26)	2.50 (1.74)
	JD-6	24.78	53.33 (46.92)	55.55 (48.20)	0.62 (1.06)	1.28 (1.34)	2.43 (1.72)
	NDRE-22	28.89	51.11 (45.64)	51.11 (45.64)	0.30 (0.89)	0.90 (1.18)	2.30 (1.67)
T_2	Local Yella	24.11	51.11 (45.64)	53.33 (46.92)	0.58 (1.04)	1.40 (1.38)	2.50 (1.74)
	Kranti	28.22	51.11 (45.64)	57.78 (49.52)	0.40 (0.95)	1.02 (1.23)	2.30 (1.67)
	JD-6	27.22	46.67 (43.08)	46.67 (43.08)	0.35 (0.92)	0.85 (1.16)	2.28 (1.67)
	NDRE-22	29.44	38.55 (39.19)	44.45 (41.80)	0.22 (0.84)	0.78 (1.13)	2.08 (1.60)
T_3	Local Yella	27.11	46.67 (43.08)	46.67 (43.08)	0.35 (0.92)	0.88 (1.18)	2.33 (1.69)
	Kranti	29.22	44.44 (41.75)	48.89 (44.36)	0.25 (0.87)	0.85 (1.16)	2.27 (1.66)
	JD-6	28.78	33.33 (35.19)	33.33 (35.19)	0.28 (0.89)	0.78 (1.13)	2.22 (1.65)
	NDRE-22	29.67	6.67 (12.45)	11.11 (19.26)	0.18 (0.82)	0.70 (1.09)	1.72 (1.49)
T_4	Local Yella	22.78	57.78 (49.53)	57.78 (52.09)	0.68 (1.09)	1.50 (1.41)	2.82 (2.33)
	Kranti	24.89	46.67 (46.92)	62.22 (52.13)	0.67 (1.08)	1.32 (1.35)	2.65 (1.78)
	JD-6	24.00	48.89 (44.36)	51.11 (45.64)	0.65 (1.07)	1.30 (1.34)	2.50 (1.74)
	NDRE-22	26.22	46.67 (43.04)	48.89 (44.36)	0.63 (1.06)	1.20 (1.30)	2.37 (1.70)
T_5	Local Yella	23.89	48.89 (44.36)	51.11 (45.64)	0.62 (1.06)	1.43 (1.39)	2.58 (1.76)
	Kranti	27.78	48.89 (44.36)	51.11 (45.64)	0.48 (0.10)	1.22 (1.31)	2.37 (1.70)
	JD-6	26.89	44.44 (41.75)	44.44 (41.75)	0.38 (0.94)	0.88 (1.18)	2.33 (1.68)
	NDRE-22	28.78	35.44 (37.05)	36.44 (37.05)	0.37 (0.93)	0.88 (1.18)	2.28 (1.67)
T_6	Local Yella	26.11	44.44 (41.75)	44.44 (41.75)	0.45 (0.97)	1.15 (1.28)	2.40 (1.71)
	Kranti	29.11	40.00 (39.19)	44.45 (41.80)	0.35 (0.92)	0.90 (1.18)	2.29 (1.68)
	JD-6	28.11	20.00 (26.36)	20.00 (26.36)	0.30 (0.89)	0.82 (1.15)	2.25 (1.66)
	NDRE-22	30.11	4.447 (10.30)	8.89 (17.12)	0.20 (0.83)	0.72 (1.10)	1.78 (1.51)
	S.Ed. (±)	0.67	4.70	3.51	0.05	0.05	0.15
	C.D. (0.05)	1.35	9.46	7.06	NS	NS	NS

Table.7 Response of genotypes on root initiation

Genotypes	Days to root initiation	Per cent (%) root initiation after			Length (cm) of regenerated root after		
		15 DAI	30 DAI	45 DAI	30 DAI	45 DAI	60 DAI
Local Yella	12.44	6.02 (2.16)	31.95 (51.11)	40.28 (58.88)	0.63 (1.07)	2.63 (1.77)	3.09 (1.89)
Kranti	12.78	6.02 (2.22)	34.72 (53.84)	43.06 (61.39)	0.66 (1.07)	2.67 (1.78)	3.11 (1.90)
JD-6	13.11	6.17 (2.35)	29.63 (49.14)	37.50 (56.43)	0.62 (1.06)	2.62 (1.77)	3.07 (1.89)
NDRE-22	13.56	4.63 (1.97)	26.86 (45.74)	35.19 (54.26)	0.60 (1.05)	2.61 (1.76)	3.06 (1.89)
S.Ed. (±)	0.41	0.29	2.12	1.99	0.01	0.01	0.01
C.D. (0.05)	0.83	NS	NS	NS	NS	NS	NS

Figures in parenthesis are transformed values.

Table.8 Performance of media with different concentrations of phytohormone for root initiation

Treatment	Treatment composition	Days to root initiation	Per cent (%) root initiation after			Length (cm) of regenerated root after		
			15 DAI	30 DAI	45 DAI	30 DAI	45 DAI	60 DAI
T_1	MS + 1 ml/l NAA + 0.5 ml/l BAP	12.97	10.42 (3.17)	37.50 (56.52)	45.83 (63.87)	0.78 (1.13)	2.82 (1.83)	3.42 (1.98)
T_2	MS + 2 ml/l NAA + 0.5 ml/l BAP	14.64	7.64 (2.78)	29.18 (48.71)	40.28 (58.90)	0.63 (1.06)	2.64 (1.77)	3.07 (1.89)
T_3	MS + 3 ml/l NAA + 0.5 ml/l BAP	16.25	2.31 (1.46)	34.03 (47.35)	37.50 (56.42)	0.54 (1.02)	2.53 (1.74)	2.93 (1.85)
T_4	B_5 + 1 ml/l NAA + 0.5 ml/l BAP	13.39	9.03 (3.07)	31.25 (53.81)	43.06 (61.39)	0.73 (1.11)	2.74 (1.80)	3.23 (1.93)
T_5	B_5 + 2 ml/l NAA + 0.5 ml/l BAP	16.06	4.86 (1.84)	31.25 (50.46)	37.50 (56.48)	0.45 (1.04)	2.59 (1.76)	2.97 (1.86)
T_6	B_5 + 3 ml/l NAA + 0.5 ml/l BAP	17.61	0.00 (0.71)	23.61 (42.91)	29.86 (49.40)	0.67 (1.01)	2.47 (1.72)	2.88 (1.85)
S.Ed. (±)		0.51	0.36	2.60	2.43	0.01	0.01	0.01
C.D. (0.05)		1.02	0.72	5.23	4.89	0.03	0.02	0.02

Table.9 Effect of genotype × treatment on root initiation

Treatment	Genotypes	Days to root initiation	Per cent (%) root initiation after			Length of regenerated root after		
			15 DAI	30 DAI	45 DAI	30 DAI	45 DAI	60 DAI
T_1	Local Yella	12.78	11.11 (3.36)	38.89 (57.76)	47.22 (65.10)	0.78 (1.13)	2.80 (1.82)	3.43 (1.98)
	Kranti	12.44	11.11 (3.00)	41.67 (60.23)	50.00 (67.5)	0.80 (1.14)	2.92 (1.85)	3.47 (1.99)
	JD-6	13.11	11.11 (3.36)	36.11 (55.36)	44.44(62. 63)	0.77 (1.13)	2.78 (1.82)	3.41 (1.98)
	NDRE-22	13.56	8.33 (2.97)	33.33 (52.73)	41.67 (60.23)	0.75 (1.12)	2.77 (1.81)	3.40 (1.98)
T_2	Local Yella	14.44	5.55 (2.22)	30.56 (49.78)	41.67 (60.23)	0.63 (1.06)	2.65 (1.78)	3.08 (1.89)
	Kranti	14.22	8.33 (2.97)	33.33 (52.73)	44.44 (62.63)	0.60 (1.07)	2.67 (1.78)	3.10 (1.90)
	JD-6	14.67	8.33 (2.97)	27.78 (47.63)	38.89 (57.60)	0.62 (1.06)	2.63 (1.77)	3.07 (1.89)
	NDRE-22	15.22	8.33 (2.97)	25.00 (44.68)	36.11 (55.13)	0.60 (1.05)	2.62 (1.77)	3.05 (1.88)
T_3	Local Yella	16.22	2.78 (1.47)	30.56 (49.78)	38.89 (57.60)	0.55 (1.03)	2.53 (1.74)	2.93 (1.85)
	Kranti	15.44	2.78 (1.47)	30.55 (50.26)	41.67 (60.23)	0.57 (1.03)	2.55 (1.75)	2.95 (1.86)
	JD-6	16.56	2.78 (1.47)	27.78 (47.63)	36.11 (55.13)	0.53 (1.02)	2.52 (1.74)	2.92 (1.85)
	NDRE-22	16.78	2.78 (1.47)	27.82 (41.73)	33.33 (52.73)	0.52 (1.01)	2.52 (1.74)	2.90 (1.84)
T_4	Local Yella	13.22	8.33 (2.98)	36.11 (54.40)	44.45 (62.70)	0.73 (1.11)	2.75 (1.76)	3.23 (1.93)
	Kranti	12.78	8.33 (2.98)	36.11 (55.36)	47.22 (65.03)	0.75 (1.12)	2.48 (1.73)	3.25 (1.94)
	JD-6	15.34	11.11 (3.37)	33.33 (52.73)	41.67 (60.23)	0.72 (1.10)	2.73 (1.80)	3.22 (1.93)
	NDRE-22	18.89	8.33 (2.98)	30.56 (52.73)	38.89 (57.60)	0.70 (1.10)	2.72 (1.80)	2.97 (1.86)
T_5	Local Yella	15.22	8.33 (2.22)	30.55 (50.26)	38.89 (57.60)	0.60 (1.05)	2.75 (1.76)	2.97 (1.86)
	Kranti	14.78	5.55 (2.22)	36.11 (54.65)	41.67 (60.23)	0.62 (1.06)	2.48 (1.73)	2.98 (1.87)
	JD-6	15.34	5.55 (2.22)	30.56 (49.78)	36.11 (55.36)	0.58 (1.04)	2.58 (1.76)	2.97 (1.86)
	NDRE-22	18.89	0.00 (0.71)	27.78 (47.15)	33.33 (52.73)	0.57 (1.03)	2.57 (1.75)	2.95 (1.86)
T_6	Local Yella	17.00	0.00 (0.71)	25.00 (44.68)	30.56 (50.10)	0.60 (1.05)	2.47 (1.72)	2.88 (1.84)
	Kranti	16.78	0.00 (0.71)	30.56 (49.78)	33.33 (52.73)	0.55 (1.03)	2.48 (1.73)	2.90 (1.84)
	JD-6	17.44	0.00 (0.71)	22.22 (41.73)	27.78 (47.63)	0.48 (0.99)	2.45 (1.72)	2.87 (1.84)
	NDRE-22	19.22	0.00 (0.71)	16.67 (35.44)	27.78 (47.15)	0.48 (0.99)	2.47 (1.72)	2.97 (1.86)
S.Ed. (±)		1.01	0.72	5.20	4.86	0.03	0.02	0.02
C.D. (0.05)		NS	NS	10.46	9.79	NS	NS	NS

Figures in parentheses are transformed values.

Plate.1 Callus initiation from anther of the genotype (A) Local Yella

Plate.2 Callus initiation from anther of the genotype (B) Kranti

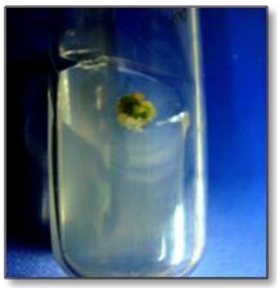


Plate.3 Shoot initiation in Local Yella

Plate.4 Shoot initiation in Kranti

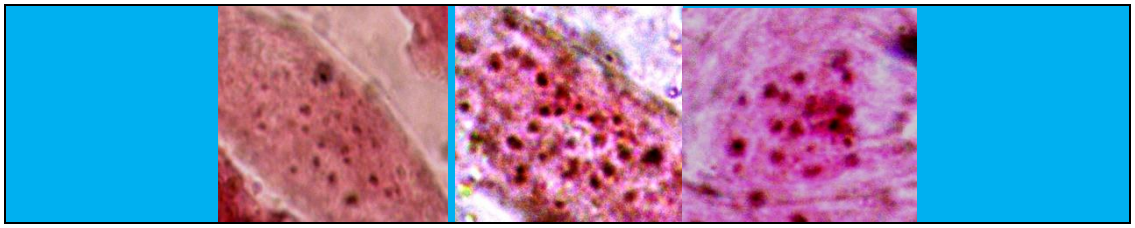
Plate.5 Shoot regeneration in Local Yella

Plate.6 Shoot regeneration in Kranti

Plate.7 Root regeneration in Local Yella

Plate.8 Root regeneration in Kranti

Plate.9 Chromosome seen under the microscope for the genotypes : (A) JD-6 (B) Kranti (C) Local Yella



Among the treatments, the media containing MS + 4 ml/l BAP + 1 ml/l NAA induced the highest percentage of calli producing shoots (55.55% and 59.44%) followed by B_5 + 4 ml/l BAP + 1 ml/l NAA (50.00% and 56.11%), B_5 + 5 ml/l BAP + 1ml/l NAA (44.42% and 36.67%), MS + 5 ml/l BAP + 1 ml/l NAA (46.86% and 50.56%) at 30 and 45 DAI respectively as shown in Table 5. This result is similar with the findings of Wang *et al.*, 2000 and Alam *et al.*, 2009. The media containing BAP at 4 ml/l induced the highest per cent of shoot initiation and it differed significantly compared to the rest of the treatments. This result is also in consonance with the finding of George *et al.*, (2008); who, revealed that BAP is most effective in promoting differentiation of cell into shoot initials followed by formation of shoot. However, the per cent of shoot initiation decreased in the media containing BAP above 5 mg/l. This is in agreement with Ravanfar *et al.*, 2009 who reported that above 5 mg/l, the mean number of shoots formed per explant decreased and became toxic to the shoot growth. In case of interaction, it was observed that per cent for shoot initiation was found to be significant indicating significant difference among the interaction. At 30 and 45 DAI, all the four genotypes (Local Yella, Kranti, JD-6 and NDRE-22) produced the highest per cent of shoot in MS + 4 ml/l BAP + 1 ml/l NAA {(60.00% and 66.67%), (57.78% and 64.44%), (53.33% and 55.55%) and (51.11% and 51.11%)} followed by B5 + 4 ml/l BAP + 1 ml/l NAA {(57.78% and 57.78%), (46.67% and 62.22%), (48.89% and 51.11%) and (46.67% and 48.89%)} respectively. Among them, Local Yella× MS + 4ml/l BAP + 1 ml/l NAA produced highest percentage of shoots (60.00% and 66.67%) followed by Kranti× MS + 4 ml/l BAP + 1 ml/l NAA (57.78% and 64.44%) at both 30 and 45 DAI; whereas, the lowest per cent of shoot was found in NDRE-22 × B5 + 6 ml/l BAP + 1 ml/l NAA (4.45% and 8.89%) followed by NDRE-22 × MS + 6 ml/l BAP + 1 ml/l NAA (6.67% and 11.11%) at both 30 and 45 DAI respectively (Table 6). This result indicated that interaction between genotype and treatment played a vital role for shoot regeneration which confirms the findings of Khan *et al.*, 2009 and Sayem *et al.*, 2010. Length of regenerated shoot was found to be significant for genotypes and treatments at 30, 45 and 60 DAI. The length of the regenerated shoot was found to be more in Local Yella at 30, 45 and 60 DAI (0.55 cm, 1.30 cm and 2.55 cm respectively). Maximum shoot length at 30, 45 and 60 DAI was observed in the media containing B5 + 4 ml/l BAP + 1 ml/l NAA (0.66 cm, 1.33 cm and 2.58 cm) followed by MS + 4 ml/l BAP + 1 ml/l NAA (0.53 cm, 1.22 cm and 2.58 cm), respectively. However, no significant variation was observed for the interaction of genotype and treatment.

Root initiation

Data on root initiation were found to be significant among genotypes for days to root initiation. Local Yella (12.44 days) took

minimum time for root initiation while NDRE-22 took maximum time (13.56 days). But the genotypes were found to be non-significant for per cent of root initiation at 15, 30 and 45 DAI and also the length of the regenerated root after 30, 45 and 60 DAI (Table 7). Significant variations were observed amongst the treatments for different parameters of root initiation like days to root initiation, per cent of root initiation at 15, 30 and 45 DAI and the length of the regenerated root after 30, 45 and 60 DAI. MS + 1 ml/l NAA + 0.5 ml/l BAP required minimum time (12.97 days) for root initiation and also produced highest percentage of root initiation in all 15, 30 and 45 DAI (10.42%, 37.50% and 45.83% respectively). The length of the regenerated root was also found to be more in this medium (0.78 cm, 2.82 cm and 3.42 cm) followed by B_5 + 1 ml/l NAA + 0.5 ml/l BAP (0.73 cm, 2.74 cm and 3.23 cm) at 30, 45 and 60 DAI respectively (Table 8).

The interaction was found to have non-significant effect for days to root initiation and length of regenerated root at 30, 45 and 60 DAI indicating that the genotypes and treatments have independent effects. Whereas the interaction of genotype × treatment showed significant variation for per cent of root initiation at 30 and 45 DAIAll the genotypes (Local Yella, Kranti, JD-6 and NDRE-22) produced maximum percentage of root in the media containing MS + 1 ml/l NAA + 0.5 ml/l BAP {(38.89%, 47.22%), (41.67%, 50.00%), (36.11%, 44.44%) and (33.33% and 41.67%)} followed by B_5 + 1 ml/l NAA + 0.5 ml/l BAP {(36.11%, 44.45%), (36.11%, 47.22%), (33.33%, 41.67%) and (30.56%, 38.89%)} at 30 and 45 DAI respectively. Among them, Kranti× MS + 1 ml/l NAA + 0.5 ml/l BAP produced the highest percentage of root (41.67%, 50.00%) followed by Local Yella× MS + 1 ml/l NAA + 0.5 ml/l BAP (38.89%, 47.22%); whereas, NDRE-22 × B_5 + 3 ml/l NAA + 0.5 ml/l BAP produced the lowest percentage of root (16.67% and 27.78%) at 30 and 45 DAI respectively (Table 9).

Cytological analysis

The root tips of the plants regenerated from the callus of anther culture were found to be haploids, that is, n= 16 (2n=36) in nature by cytological examination which confirms the findings of Keller and Armstrong (1983) and, Prabhudesai and Bhaskaran (1993) who revealed that the plants regenerated from the anther culture were haploids in nature.

From the results of this experiment, it can be concluded that among the genotypes, Local Yella was found to be best in all the cases that is, for callus induction, shoot regeneration and root initiation. Among the treatments, the media containing B_5 + 3 ml/l 2,4-D + 1 ml/l BAP and MS + 3 ml/l 2,4-D + 1 ml/l BAP performed best for callus induction. For shoot regeneration, MS + 4 ml/l BAP + 1 ml/l NAA followed by B_5 + 4 ml/l BAP + 1 ml/l NAA was found to be best and for root initiation, the media containing MS + 1 ml/l NAA + 0.5 ml/l BAP and B_5 + 1 ml/l NAA + 0.5 ml/l BAP performed best.

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