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Assessment of Callus Induction and Whole Plant Regeneration in Sub-tropical Maize (*Zea mays* L.) using Plumule as Explant

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Abstract

Callus induction and whole plant regeneration was assessed with plumule as explant derived from three genotypes VQL1, VQL2 and CM145 on MS and N6 based media. Among the 3 genotypes, the highest mean percentage of primary callus induction was recorded for CM 145 to be 97.74 ± 0.28 and 81.85 ± 0.37 on MS and N6 based media respectively. Similarly, the highest mean percentage of embryogenic callus induction was recorded for CM 145 to be 10.75 ± 0. 22 and 7.74 ± 0.20 on MS and N6 based media respectively. The highest mean whole plant regeneration for CM 145 was recorded to be 0.2 ± 0 on MS basal media among the nine different regeneration media tested. Since, the regeneration frequency was very low and sporadic, callus induction and robust whole plant regeneration was standardized with alternative explant.

***Key words*:** Sub-tropical maize, CM145, Mature embryo, Plumule, Somatic embryo

Maize (*Zea mays* L.) is one of the three most extensively cultivated cereal crops is crucial to feeding and sustaining the world's expanding population [1]. It serves as a source of food, feed, biofuel and raw materials for manufacturing industrial materials. It is grown in Argentina, Brazil, China, Hungary, Indonesia, Italy, Mexico, Philippines, South Africa, Romania, United States and Yugoslavia in addition to India. In India, the maize farming has increased over time as a result of ongoing efforts however, 45 million tonnes of maize must be produced by 2030 to meet the growing demands. Horizontal growth by way of area is challenging, therefore it's important to investigate ways to increase productivity by comprehending the production limits. Among all the limitations, a variety of biotic and abiotic stressors that limit maize production are a significant factor in lowering the productivity of maize [2-3]. In India, the higher yield of maize is severely affected by biotic factors like stem borer infestations. The gene pool of maize lacks the sources of resistance to the stem borer. Therefore, developing Bt-protected maize in India is justified owing to the prevalence of stem borer infestation and its related negative consequences on maize productivity [4]. The availability of an effective *in vitro* plant regeneration and transformation technology is a crucial necessity for the development of transgenic plants. Though, development of transgenic maize has been reported in genotypes adapted to temperate zones using immature embryos however, maize regeneration and transformation in genotypes adapted to tropical and subtropical conditions using mature embryos as explants have been scarce [5]. Regular supply of immature embryos is a challenge, since the chosen genotypes must be sown at regular intervals of time. Besides, that environmental factors have a significant impact on the immature embryos used as explant. Considering the facts, the assessment of callus induction and whole plant regeneration was carried out using plumule (mature embryo) as explant.

MATERIALS AND METHODS

The healthy seeds of VQL 1, VQL 2 and CM 145 were selected and treated with 1% Bavistin (w/v) for 20-30 min to disinfect. Following Bavistin treatment, seeds were washed with sterile H_2O for 4-5 times. Then, Bavistin-treated seeds were again treated with 0.2% (w/v) SDS and 0.1% (w/v) $HgCl_2$ for 10 min, followed by washing with sterile dd H_2O for 4 times. To cut plumules, seeds were soaked in sterile dd H_2O containing 4 mg l^{-1} 2,4-D for 48 h, then inoculated on MS medium and N6 medium containing 3 mg l^{-1} 2, 4-D for 48 hours. The excised plumule sections (3-4 mm) were sliced half lengthwise and plated facing the cut surface on the following callus induction media:

(a). MS salts [6] and vitamins supplemented with 1 mg l^{-1} 2, 4-D, 3% (w/v) sucrose and 0.3% (w/v) clarigel, pH 5.8 (CIM1).

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b). MS salts [6] and vitamins supplemented with 2 mg l^{-1} 2, 4-D, 3% (w/v) sucrose and 0.3% (w/v) clarigel, pH 5.8 (CIM2).
c). MS salts [6] and vitamins supplemented with 3 mg l^{-1} 2, 4-D, 3% (w/v) sucrose and 0.3% (w/v) clarigel, pH 5.8 (CIM3).
d). MS salts [6] and vitamins supplemented with 4 mg l^{-1} 2, 4-D, 3% (w/v) sucrose and 0.3% (w/v) clarigel, pH 5.8 (CIM4).
e). N6 salts [7] and vitamins supplemented with 1 mg l^{-1} 2, 4-D, 3% (w/v) sucrose and 0.3% (w/v) clarigel, pH 5.8 (CIM5).
f). N6 salts [7] and vitamins supplemented with 2 mg l^{-1} 2, 4-D, 3% (w/v) sucrose and 0.3% (w/v) clarigel, pH 5.8 (CIM6).
g). N6 salts [7] and vitamins supplemented with 3 mg l^{-1} 2, 4-D, 3% (w/v) sucrose and 0.3% (w/v) clarigel, pH 5.8 (CIM7).
h). N6 salts [7] and vitamins supplemented with 4 mg l^{-1} 2, 4-D, 3% (w/v) sucrose and 0.3% (w/v) clarigel, pH 5.8 (CIM8).

Primary calli induced after 15 days of culture on MS and N6 medium (cycle I) were evaluated and cultured on MS medium (CIM9) and N6 medium (CIM10) respectively, supplemented with 2 mg l^{-1} 2, 4-D and 2 mg l^{-1} NAA for 15 days (cycle II). At the end of the second cycle, calli from CIM9 and CIM10 medium were selected and cultured on MS medium (CIM11) and N6 medium (CIM12) supplemented with 1 mg l^{-1} 2, 4-D and 0.3 mg l^{-1} NAA for the next 15 days (cycle III). All the steps of callus formation were performed in the dark at 25°C. After two biweekly cultures, the embryogenic calli derived from MS and N6-based media were evaluated in more detail.

Plant regeneration

Actively growing calli induced from embryos derived from CM 145 were selected and cultured on regeneration medium. After the shoots and roots were obtained in a Petri dish, the seedlings were transferred to a Magenta box containing half strength MS medium to induce multiple roots, followed by hardening of the regenerated plants in the greenhouse.

RESULTS AND DISCUSSION

Induction of primary calli from plumule on MS and N6 based medium

Four different MS based (CIM1, CIM2, CIM3 and CIM4) and N6-based media (CIM5, CIM6, CIM7 and CIM8) were used to develop primary callus from inoculated plumules derived from 3 genotype viz., VQL1, VQL 2, CM 145. Different responses to callus formation were observed among 3 genotypes on 4 different callusing media. Based on ANOVA, the DMRT test was performed at 5% significance level to compare the rate of primary calli development on 4 different media based on MS and N6. The mean rate of primary callus induction on MS-based medium ranged from 19.98 ± 0.28 to 97.74 ± 0.28, and the mean rate of primary callus induction on N6-based media ranged from 15.08 ± 0.19 to 81.85 ± 0. 37 depending on genotype and 2, 4-D concentration. Among the 3 genotypes, CM 145 showed the highest mean primary callus formation, followed by VQL 2 and VQL 1 on MS and N6 medium (Fig 1-2), (Tables 1-2).

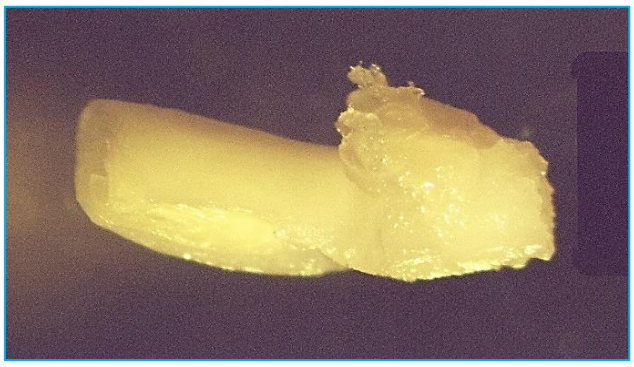

Fig 1 Primary callus induction from plumule derived from CM145 on MS based medium (CIM 4) containing 4 mg/l,2 4-D

Fig 2 primary callus induction from plumule derived from CM145 on N6 based medium (CIM 8) containing 4 mg/l,2 4-D

Induction of embryogenic calli on MS and N6 based medium

Primary calli were transferred to fresh cultures containing different concentrations of 2,4-D and NAA. After two biweekly cultures, calli were classified into two types. One category consisted of non-embryogenic, soft, watery, brown callus; the other consisted of compact, irregularly shaped, cream-like embryogenic callus. Based on ANOVA (Analysis of variance), the Duncan's Multiple Range Test *(*DMRT*)* test was performed at 5% significance level to compare the mean percentage of embryogenic callus induction among the three genotypes. The mean percentage induction of embryogenic callus on MS medium varied significantly ($p < 0.05$) between the 3 genotypes and was maximum recorded for CM 145 (10.75 ± 0. 22), followed by VQL 2 (5. 95 ± 0. 23) and VQL 1 (4.01 ± 0. 16) and the mean percentage of embryogenic calli on N6-based media ranged from a maximum for CM 145 (7. 74 ± 0. 20), followed by VQL 2 (3. 86 ± 0. 20) and VQL 1 (2. 49 ± 0. 09) (Tables 3-4).

Plant regeneration

CM 145 derived embryogenic calli were cultured on nine regenerative media formulations i.e., MS + Kin (0.1), MS + Kin (0.2), MS + BAP (0.1), MS + BAP (0.2), MS + BAP (3.5), MS + Kin (3.5), MS + BAP (3.5) + NAA (0.5), MS + Kin (3.5) + NAA (0.5) and basal MS [6]. Based on ANOVA, the DMRT test was performed at 5% significance level to compare the percentage of greening and percentage of whole plant regeneration of embryogenic calli on different regeneration media. Among the 9-regeneration media, the highest mean greening rate of embryogenic calli was recorded at 9. 48 ± 0. 28 on basal MS and the lowest mean greening rate was recorded at 4.14 ± 0. 02 on MS + Kin (3. 5) + NAA (0. 5), while the average whole plant regeneration percentage ranged from 0.04 to 0.2. Among the 9 regeneration medium formulations, the basal MS was found to be the best for whole plant regeneration (0. 2 ± 0), which was significantly higher ($p < 0.05$) than the other regeneration medium (Tables 5-6).

Table 1 Effects of 2, 4-D on induction of primary calli after 15 days

Percentage of primary calli development [1,2]				
MS based medium				
Genotypes	CIM1	CIM2	CIM3	CIM4
VQL1	19.98 ± 0.28 (26.57)^{c}	62.80 ± 0.61 (52.45)^{b}	79.61 ± 0.51 (63.19)^{a}	80.36 ± 0.46 (63.73)^{a}
VQL2	28.25 ± 0.35 (32.12)^{c}	82.40 ± 0.19 (65.23)^{b}	93.91 ± 0.4 (75.77)^{a}	94.71 ± 0.39 (76.77)^{a}
CM145	32.97 ± 0.46 (35.06)^{c}	82.40 ± 0.31 (68.26)^{b}	97.31 ± 0.30 (80.64)^{a}	97.74 ± 0.28 (81.43)^{a}

1. Data represents the means (± SE) of three replicates, each with 200 spliced plumules.
2. Percentage values were subjected to arcsine transformation before analysis. A value in parenthesis is transformed value
$^{a-c}$ Values in parenthesis followed by different letters in a raw are significantly different at p<0.05 according to Duncan's multiple range test

Table 2 Effects of 2, 4-D on induction of primary calli after 15 days

Percentage of primary calli development [1, 2]				
N6 based medium				
Genotypes	CIM1	CIM2	CIM3	CIM4
VQL1	15.08 ± 0.19 (22.86)^{c}	50.74 ± 0.48 (45.45)^{b}	68.68 ± 0.49 (56.0)^{a}	69.36 ± 0.65 (56.42)^{a}
VQL2	20.51 ± 0.28 (26.94)^{c}	69.96 ± 0.32 (56.79)^{b}	75.34 ± 0.32 (60.26)^{a}	76.11 ± 0.28 (60.77)^{a}
CM145	24.45 ± 0.41 (29.65)^{c}	73.62 ± 0.28 (59.13)^{b}	81.11 ± 0.31 (64.28)^{a}	81.85 ± 0.37 (64.82)^{a}

1. Data represents the means (± SE) of three replicates, each with 200 spliced plumules.
2. Percentage values were subjected to arcsine transformation before analysis. A value in parenthesis is transformed value
$^{a-c}$ Values in parenthesis followed by different letters in a raw are significantly different at p<0.05 according to Duncan's multiple range test

Induction of primary calli from plumule on MS and N6 based medium

In an attempt to systematically study callus induction and plant regeneration in the maize genotypes VQL 1, VQL 2, and CM 145 different concentrations of 2, 4-D and other plant growth regulators were added onto both MS and N6 media. Spliced plumules from the VQL 1, VQL 2, and CM 145 genotypes were used to induce primary calli on MS-based (CIM1, CIM2, CIM3, and CIM4) and N6-based (CIM5, CIM6, CIM7, and CIM8) media. In all 3 genotypes, it was found that the callus induction medium composition had an impact on how frequently primary calli were induced. CIM4 (a medium containing 4 mg l^{-1} 2, 4-D) was shown to had the highest induction of primary calli among the four MS-based medium. Similarly, among the 4 N6-based media studied, CIM8 (a medium containing 4 mg l^{-1} 2, 4-D for all 3 genotypes) showed the highest induction of primary calli. High concentration of 2, 4-D (4 mg l^{-1}) in the medium are likely to be reason for the increased callus induction percentages in CIM4 compared to CIM3, CIM2, CIM1, and CIM8 compared to CIM7, CIM6, and CIM5, respectively. This finding is in line with earlier research that showed the importance of 2, 4-D for the initiation and proliferation of primary calli from maize embryos at various developmental stages, including immature [8-9], mature [10], and immature and mature [11-12]. Similar findings were made by Vikrant and Rashid [13] (2002), who showed that 2, 4-D is essential for the development of primary and embryogenic calli in cereals. The current study found no significant differences between MS-based medium, CIM4 (medium containing 4 mg l^{-1} 2, 4-D), and CIM3 (medium containing 3 mg l^{-1} 2, 4-D) in terms of inducing amount and quality of calli for all three genotypes. Similarly, two N6-based medium, CIM8 (medium containing 4 mg l^{-1} 2, 4-D) and CIM7 (medium containing 3 mg l^{-1} 2, 4-D), both showed a similar tendency. For callus induction, CIM3 and CIM7 were more effective than CIM4 and CIM8. The success of CIM3 and CIM7 for inducing calli over CIM4 and CIM8 is in line with Huang and Wei's [10] earlier study, which found that a higher concentration of 2,4-D had no appreciable impact on either the quantity or quality of calli that were induced. Because higher concentrations of 2, 4-D may cause somatic mutation [14] or have an impact on plant regeneration [15]. Similarly, Ombori *et al*. [16] reported the development of primary and embryogenic calli were inhibited by increased concentrations of 2, 4-D. Ombori *et al*. [16] argued that higher concentration of 2,4-D affects cell division and this may be the cause of less induction of primary and embryogenic calli. So medium containing 3 mg l^{-1} 2, 4-D (CIM3 and CIM7) were chosen as the preferred medium for induction of primary calli. CM 145 showed the highest rate of primary calli induction among the three genotypes, regardless of the type of media utilized (MS and N6 based media). This finding is consistent with the findings of several researchers, including Bohorova *et al*. [9], Furini and Jewell [17], and Aguado-Santacruz *et al*. [18], who showed that genotypes are crucial for the induction of callus in maize.

Induction of embryogenic calli on MS and N6 based medium

The induction of embryogenic callus and the maintenance of embryogenesis are important prerequisites for a robust regenerative system. Of the three genotypes, viz. VQL 1, VQL 2 and CM 145, the highest percentage of embryogenic calli was recorded for CM 145 on MS and N6 medium. Primary calli cultured on medium containing 2,4-D (2.0 mg l^{-1}) and NAA (2.0 mg l^{-1}) for 15 days and then transferred the calli to low dose 2,4-D (1. 0 mg l^{-1}) and NAA (0.3 mg l^{-1}) for another 15 days resulted in embryogenic callus formation. This observation is consistent with Agrawal *et al*. [16] that the sequential reduction of 2,4-D enhances long-term maintenance of whole plant regeneration from callus in some indica rice varieties. In addition, among the auxins, 2-,4-D and NAA have been shown to be beneficial for callus induction in cereals, as observed by Zhu *et al*. [19]. Similarly, the synergy of 2, 4-D and NAA for good quality callus induction was observed in *Achyranthes aspera* L [20]. The results obtained from the present study showed a difference in embryogenic callus induction on MS and N6-based media. The induction of embryogenic calli was found to be higher on MS-based media than in N6-based media, which is in contrast to the observation by Bohorova *et al*. [9], Rakshit *et al*. [21] that the N6 medium was better than the MS medium. Bohorov *et al*. [9] observed that lower nitrogen levels in N6 medium were better for callus formation and maintenance than in MS medium. Contrary to the observations of Bohorova *et al*. [9], Rakshit *et al*. [21], Biswas and Mandal [22] observed that the MS environment was the best among the three. MS, LS and N6 were tested for callus formation, seedling regeneration and number of seedlings/seed

callus in rice. These observations are similar to the results obtained in this study.

Table 3 Induction of embryogenic calli on MS based medium

Genotypes	Percentage of embryogenic calli development [1,2]
VQL 1	4.01 ± 0.16 (11.54)[c]
VQL2	5.95 ± 0.23 (14.11)[b]
CM145	10.75 ± 0.22 (19.14)[a]

1: Data represents the means (± SE) of three replicates, each with 150 primary callus
2: Percentage values were subjected to arcsine transformation before analysis. A value in parenthesis is transformed value
[a-c]Values in parenthesis followed by different letters differ significantly at p<0.05 according to Duncan's multiple range test

Table 4 Induction of embryogenic calli on N6 based medium

Genotypes	Percentage of embryogenic calli development [1,2]
VQL 1	2.49 ± 0.09 (9.08)[c]
VQL2	3.86 ± 0.20 (11.31)[b]
CM145	7.74 ± 0.20 (16.15)[a]

1: Data represents the means (± SE) of three replicates, each with 150 primary callus
2: Percentage values were subjected to arcsine transformation before analysis. A value in parenthesis is transformed value
[a-c]Values in parenthesis followed by different letters differ significantly at p<0.05 according to Duncan's multiple range test

Table 5 Response of regeneration media

Regeneration media	Greening percentage [1,2]
MS + Kin (0.1)	5.96 ± 0.09 (14.14)[e]
MS + Kin (0.2)	5.18 ± 0.08 (13.16)[f]
MS + BAP (0.1)	6.60 ± 0.15 (14.89)[d]
MS + BAP (0.2)	7.21 ± 0.08 (15.58)[c]
MS + BAP (3.5)	8.26 ± 0.02 (16.71)[b]
MS + Kin (3.5)	7.40 ± 0.12 (15.79)[c]
MS + BAP (3.5) + NAA (0.5)	6.41 ± 0.15 (14.67)[d]
MS + Kin (3.5) + NAA (0.5)	4.14 ± 0.02 (11.75)[g]
MS Basal	9.48 ± 0.28 (17.93)[a]

1: Data represents the means (± SE) of three replicates, each with 150 primary callus
2: Percentage values were subjected to arcsine transformation before analysis. A value in parenthesis is transformed value
[a-c]Values in parenthesis followed by different letters differ significantly at p<0.05 according to Duncan's multiple range test

Plant regeneration

In vitro regeneration of maize calli has been reported through embryogenesis [23-27]. Vasil *et al*. [28] argued that embryogenesis is the most common regeneration pathway in small tissues proliferating from the immature maize embryos, as revealed by histological studies. Several factors such as embryo size, genotype and growth regulators present in the culture medium are known to influence the in vitro regeneration of maize tissues [29-32]. When CM 145-derived embryogenic callus was introduced into nine different MS-based regeneration systems, i.e., MS+Kin (0.1), MS+Kin (0.2), MS+BAP (0.1), MS+BAP (0.2), MS+BAP (3.5), MS + Kin (3.5), MS + BAP (3.5) + NAA (0.5), MS + Kin (3.5) + NAA (0.5) and basal MS [6], the highest percentage of whole plant regeneration was obtained on MS basal medium. The results showed that the use of hormones was not necessary for the regeneration of maize plants, which is consistent with the reports of various researchers [10], [33]. A possible explanation for this phenomenon was given by Huang and Wei [10], who suggested that somatic embryos capable of producing new seedlings were already formed and their fate could be predetermined by the environment. Typically, the ability of callus' to regenerate plants correlates with its ability to form embryonic callus. However, in the present investigation, the observed trend is different. Most embryogenic calli typically produce secondary and tertiary embryos instead of regenerating into whole plantlets, as demonstrated by very low whole plant regeneration rates in the nine different regeneration media tested. This means that although the callus is embryogenic, the composition of the medium is not suitable enough to activate the functional components of metabolism responsible for the regeneration of the entire plant. Because the regeneration frequency of the embryogenic callus was too low for his ability to form whole plant and to be used in any transgenic program, callus induction and whole plant regeneration have been eliminated. Instead, the second method of callogenesis and regeneration of whole plant using the spliced nodal segments was attempted [2].

Table 6 Response of regeneration media

Regeneration media	Whole plant regeneration percentage[1,2]
MS + Kin (0.1)	0.04 ± 0 (0.02)[e]
MS + Kin (0.2)	0.04 ± 0 (0.02)[e]
MS + BAP (0.1)	0.13 ± 0.01 (0.05)[c]
MS + BAP (0.2)	0.13 ± 0.01 (0.05)[c]
MS + BAP (3.5)	0.16 ± 0.(0.06)[b]
MS + Kin (3.5)	0.12 ± 0 (0.05)[c]
MS + BAP (3.5) + NAA (0.5)	0.12 ± 0 (0.05)[c]
MS + Kin (3.5) + NAA (0.5)	0.08 ± 0 (0.03)[d]
MS Basal	0.2 ± 0 (0.08)[a]

1: Data represents the means (± SE) of three replicates, each with 150 primary callus
2: Percentage values were subjected to arcsine transformation before analysis. A value in parenthesis is transformed value
[a-c]Values in parenthesis followed by different letters differ significantly at p<0.05 according to Duncan's multiple range test

CONCLUSION

Callus induction and whole plant regeneration of sub-tropical maize genotypes VQL1, VQL2 and CM 145 was evaluated on MS and N6 based media using plumule as explant. Among the genotypes tested, callus induction was found to be highest for CM 145 on MS based media and among the nine different regeneration media tested whole plant regeneration was achieved on MS basal media. Although, very low percentage and sporadic whole plant regeneration was recorded however, the study holds importance since somatic embryogenesis of maize using mature embryo as explant is limited. Further refinement of induction of embryogenic callus and screening of more number of genotypes may lead to robust whole plant regeneration.

LITERATURE CITED

1. Wang AS. 1987. Callus induction and plant regeneration from maize mature embryos. *Plant Cell Reproduction* 6(5): 360-362.

2. Tiwari S, Agrawal PK, Pande V, Gupta HS. 2015. Callus induction and whole plant regeneration in sub-tropical maize (*Zea mays* L.) using mature embryos as explants. *Indian Journal of Genetics and Plant Breeding* 75(3): 330-335. https://doi.org/10.5958/0975-6906.2015.00052.8
3. Manivannan A, Kaul J, Singode A, Dass S. 2010. Callus induction and regeneration of elite Indian maize inbreds. *African Journal of Biotechnology* 9(44): 7446-7452.
4. Wenbin L, Masilamany P, Kasha KJ, Pauls P. 2002. Development, tissue culture and genotypic factors affecting plant regeneration from shoot apical meristem of germinated *Zea mays* seedlings. *In vitro cellular and Developmental Biology* 38: 285-292.
5. Malini N, Ananadakumar CR, Ramakrishnan SH. 2015. Regeneration of Indian maize genotypes (Zea mays L.) from immature embryo culture through callus induction. *Journal of Applied and Natural Science* 7(1): 131-137.
6. Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiology Plant* 15: 473-497.
7. Chu CC, Wang CC, Sun CS, Hsu C, Yin KC, BI CV. 1975. Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen source. *Sci. Sin.* 18: 659-668.
8. Carvalho CHS, Bohorova N, Bordallo PN, Abreu LL, Valicentle FH, Bressan W, Paiva E. 1997. Type II callus production and plant regeneration in tropical maize genotypes. *Plant Cell Rep*. 17: 73-76.
9. Bohorova NE, Luna B, Briton RM, Huerta LD, Hoistington DA. 1995. Regeneration potential of tropical, and subtropical, mid-altitude, and highland maize inbreds. *Maydica* 40: 275-281.
10. Huang XO, Wei ZM. 2004. High frequency plant regeneration through callus initiation from mature embryos of maize (*Zea mays* L.). *Plant Cell Rep*. 22: 793-800.
11. Bi RM, Kou M, Chen LG, Mao SR, Wang HG. 2007. Plant regeneration through callus initiation from mature embryo of *Triticum*. *Plant Breeding* 126: 9-12.
12. Yu GR, Yin J, Guo TC, Niu JS. 2003. Selection of the optimum genotype for immature embryo culture of wheat. *Journal of Triticeae Crops* 23: 14-18.
13. Vikrant A, Rashid A. 2002. Somatic embryogenesis from immature and mature embryos of a minor millet *Paspalum scrobiculatum* L. *Plant Cell Tissue Organ Culture* 69: 71-77.
14. Choi H, Lemaux PG, Cho M. 2001. Selection and osmotic treatment exacerbate cytological aberrations in transformed barley (*Hordeum vulgare* L.). *Journal of Plant Physiology* 158: 935-943.
15. Agrawal PK, Gosal SS, Sidhu GS. 2006. Sequential reduction of 2, 4-D improves whole plant regeneration from long-term maintained calli in some indica cultivars of rice (*O. sativa* L.). *Oryza* 43: 10-15.
16. Ombori O, Gitonga NM, Machuka S. 2008. Somatic embryogenesis and plant regeneration from immature embryos of tropical maize (*Zea mays* L.) inbred lines. *Biotechnology* 7: 224-232.
17. Furini A, Jewell DC. 1994. Somatic embryogenesis and plant regeneration from immature and mature embryos of tropical and subtropical *Zea mays* L. genotypes. *Maydica* 39: 155-164.
18. Aguado-Santacruz GA, Garcia-Moya E, Aguilar-Acuna JL, Moreno-Gomez B, Solis-Moya E, Preciado-Ortiz ER, Jimenez-Bremont JF, Rascon-Cruz Q. 2007. In vitro plant regeneration from quality protein maize (QPM*). In Vitro Cell and Dev. Biol Plant* 43: 215-224.
19. Zhu DY, Sun ZX, Pan XG, Ding XH, Shen XH, Won Y, Pan H, Yin JH, Alejar MS, Torrizo LB, Datta SK. 1998. Use of anther culture in hybrid rice breeding. *Proceedings of the 3rd International Symposium of Hybrid Rice* 14–16 Nov 1996. Hyderabad, India. *In*: Advances in Hybrid Rice Technology. International Rice Research Institute, Manila Philippines 21: 265-281.
20. Sen MK, Nasrin S, Rahman S, Jamal AHM. 2014. In vitro callus induction and plantlet regeneration of *Achyranthes aspera* L., a high value medicinal plant. *Asian Pac. Jr. Trop. Biomed*. 4: 40-46.
21. Rakshit S, Rashid Z, Sekhar JC, Fatma T, Dass S. 2010. Callus induction and whole plant regeneration in elite Indian maize (*Zea mays* L.) inbreds. *Plant Cell Tissue Organ Culture* 100: 31-37.
22. Biswas A, Mandal AB. 2007. Plant regeneration in different genotypes of indica rice. *Indian Jr. of Biotechnolgy* 6: 532-540.
23. Green CE. 1982. Somatic embryogenesis and plant regeneration from the friable callus of *Zea mays*. *In*: (Eds) Fujiwara A. Plant Tissue Culture. Proceedings of the 5th International Congress Plant Tissue and Cell Culture. Tokyo, Japan. pp 107-108.
24. Lu C, Vasil IK, Ozias-Akins P. 1982. Somatic embryogenesis in *Zea mays* L. *Theor. Appl. Genetics* 62: 109-112.
25. Lu C, Vasil V, Vasil IK. 1983. Improved efficiency of somatic embryogenesis and plant regeneration in tissue cultures of maize (*Zea mays* L.). *Theor. Appl. Genetics* 66: 285-289.
26. Novak FJ, Dolezelova M, Nesticky M, Piovarci A. 1983. Somatic embryogenesis and plant regeneration in *Zea mays* L. *Maydica* 28: 381-390.
27. Lowe K, Taylor DB, Ryan P, Paterson KE. 1985. Plant regeneration via organogenesis and embryogenesis in maize inbred line B73. *Plant Science* 41: 125-132.
28. Vasil V, Lu CY, Vasil IK. 1985. Histology of somatic embryogenesis in cultured immature embryos of maize (*Zea mays* L.). *Protoplasma* 127: 1-8.
29. Green CE, Philips RL. 1975. Plant regeneration from tissue cultures of maize. *Crop Science* 15: 417-421.
30. Duncan DR, Williams ME, Zehr BE, Widholm JM. 1985. The production of callus capable of plant regeneration from immature embryos of numerous *Zea mays* (L.) genotypes. *Planta* 165: 322-332.
31. Tomes DT, Smith OS. 1985. The effects of parental genotype on isolation of embryogenic callus from elite maize germplasm. *Theor. Appl. Genetics* 70: 505-509.
32. Hodges TK, Kamo KK, Imbrie CW, Beckwar MR. 1986. Genotype specificity of somatic embryogenesis and regeneration in maize. *Biotechnology* 4: 219-223.
33. Armstrong CL, Green CE. 1985. Establishment and maintenance of friable, embryogenic maize callus and involvement of L-Proline. *Planta* 164: 207-214.