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SHORT COMMUNICATION

IN VITRO FLOWERING IN HILL MAIZE: A NOVEL TECHNIQUE FOR FUTURE

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***In vitro* flowering through shoot tip culture was analyzed in four maize genotypes, *viz*. Vivek-4, Vivek-5, VL-42 & Him-129. Plants were regenerated from first nodal region of shoot tip in MS medium supplemented with 2 mg/l BAP and 500 mg/l casein hydrolysate. Multiple shooting was observed in all these varieties. After six week (42 days) of culture miniature cobs were developed only in three genotypes, i.e. Vivek-5, VL-42 and Him-129, under 16 hr photoperiod and 150 µmol m^{-2} sec^{-1} light intensity at 25±2°C. *In vitro* flowering may be used as an effective tool for advancing hybrid production and to study the physiological and molecular aspects of flowering in maize.**

Key words: In vitro* flowering, shoot tip culture, *Zea mays

Maize is the third most important crop in the world after rice and wheat cultivated over an area of about 145 million hectares, with an annual production of 779.6 million tones. Developing countries account for 64% of the world's maize area and 43% of global maize production (CIMMYT 2008). Maize is grown over an area of 7.6 million hectares in India, with total production of 14.7 million tones (Anonymous 2008). Though, there is large area (38,635 hectare) under maize in Uttarakhand, but the productivity is low (14.752 t/ha) thus there is tremendous scope for maize improvement in Uttarakhand.

Flowering is a complex process, governed by external and internal factors and its induction under *in vitro* culture is rare (Anitha and Kumari 2006). It could be of immense importance in selective hybridization especially using pollens from rare stocks, due to high genetic purity (Stephan and Jayabalan 1998). It widens the understanding of physiology of flowering and depends primarily upon the level and interaction of exo- and endogenous phytohormones, sugars, minerals, phenolics, quality, quantity and length of light and temperature during *in vitro* culture (Tanimoto and Harada 1981a, b). *In vitro* flowering was previously reported in citrus (Moss 1969), cauliflower (Kumar *et al*. 1995), coriander (Stephan and Jayabalan 1998), bamboo (Singh *et al*. 2000), tomato (Sheeja and Mandal 2003), *Brassica* (Verma and Singh 2007) and *Withania somniferum* (Ashwagandha) (Saritha and Naidu 2007). *In vitro* flowering and fruiting may significantly contribute to genetic improvement of maize involving rare stocks, not accessible through conventional plant breeding. The objective of this study was to analyze *in vitro* flowering potential of maize genotypes from Uttarakhand through shoot tip culture. The present communication reports successful *in vitro* flowering.

Seeds of four cultivars of maize from hills namely, Vivek-4, Vivek-5, VL-42 and Him-129 were obtained from Vivekananda Parvatiya Krishi Anusandhan Sansthan (ICAR), Almora. Mature seeds of maize

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genotypes were surface sterilized in 0.1% $HgCl_2$ solution supplemented with 2-3 drops of detergent for 2 minutes. To remove mercuric chloride, washing was performed 5 times with sterilized distilled water. To facilitate germination, seeds were place with embryo side up in the MS medium in 450 ml glass bottles (Jam bottles) and were maintained at 28±2°C in B.O.D incubator.

For culture initiation, Murashige and Skoog (Murashige and Skoog 1962) medium supplemented with 2 mg/l BAP and 500 mg/l casein hydrolysate along with 3% sucrose, 0.6% agar and pH adjusted to 5.8 (autoclaved at 15 psi for 20 minutes) was used. Five days old 3-5 cm long sections of shoot tips were excised from the 1st nodal region and placed horizontally in the medium (Fig. 1A). Cultures were maintained in tissue culture chamber at 16 hr photoperiod, 150 µmol m^{-2} sec^{-1} light intensity and 25±2°C temperature. The proliferated plants were sub cultured after 21 days interval in the MS medium supplemented with 2 mg/l BAP and 500 mg/l casein hydrolysate. Sub culturing was again performed to place definite shoots onto the liquid medium of same composition. Data were analyzed using standard statistical methods.

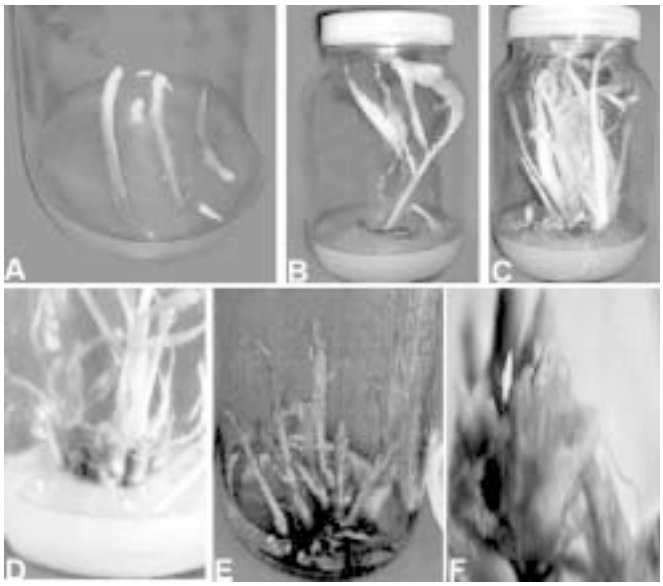

Fig. 1. *In vitro* cob induction from shoot tip culture in maize. (A) Inoculation of 5 days old shoot tips induced on MS with 2 mg/l BAP and 500 mg/l casein hydrolysate (B) Shoot induction on MS with 2 mg/l BAP and 500 mg/l casein hydrolysate (C) Multiple shoot induction in subculturing under *in vitro* culture (D) Miniature cob induction in solid MS medium with 2 mg/l BAP and 500 mg/l casein hydrolysate (E) Miniature cob development in liquid MS medium with 2 mg/l BAP and 500 mg/l casein hydrolysate (F) Fully bloomed flowers under *in vitro* culture

In vitro cultured shoot tips elongated after one week and their multiplication started after 15 days (Fig. 1A-C). During the initial three weeks of subculture, explants did not show any significant variation in number of leaves (Fig. 2) and shoots (Fig. 3) among different genotypes of maize. The effective genotypic variation in number of leaves and shoots was observed in first and second subculture. Vivek-5 had the highest number of leaves

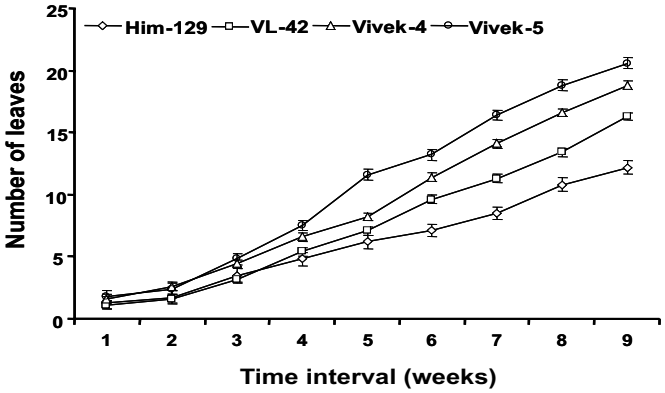

Fig. 2. Average number of leaves produced during shoot tip culture of maize (*Zea mays* L.) in cultivars Him-129, VL-42, Vivek-4 and Vivek-5 in MS medium supplemented with 2mg/l BAP and 500 mg/l casein hydrolysate. Data represent the mean of 20 replicates

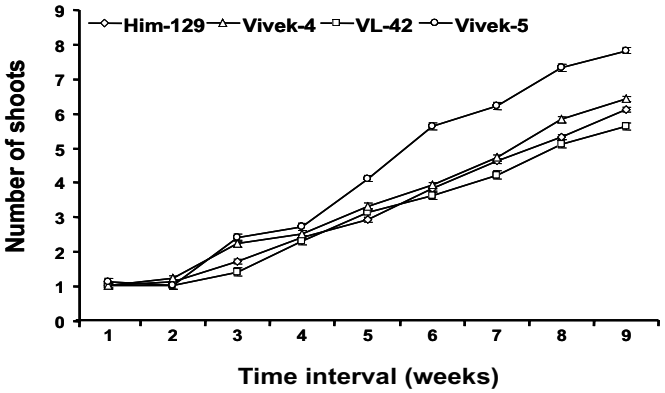

Fig. 3. Average number of shoots produced during shoot tip culture of maize (*Zea mays* L.) in cultivars Him-129, VL-42, Vivek-4 and Vivek-5 in MS medium supplemented with 2 mg/l BAP and 500 mg/l casein hydrolysate. Data represent the mean of 20 replicates

and shoots from second subculture (4th week). Production of multiple shoots with BAP alone showed the confirmation of earlier works in *Rauvolfia* (Viswanath and Jeyanthi 1997, Anitha and Kumari 2006).

After 6 weeks (42 days) of culture, miniature cobs were recorded in maize genotypes Vivek-5, VL-42 and Him-129 (Fig. 1D-F). Vivek-5 showed the maximum cobs through shoot tip cultured plantlets. Silk was also observed when the cultures were kept for prolonged sub culturing (Fig. 1E, Table 1). Vivek-5 had the highest number of cob and silk production per shoot tip culture followed by VL-42. However, Vivek-4 did not show induction of flowering in terms of cob and silk production. The reason for this variation is not clear. BAP used for induction of *in vitro* flowering played a major role in flower bud formation and is consistent with the earlier work reported in *Vitex negundo* L. (Thiruvengadam and Jayabalan 2001), *Momordica charantia* L. (Wang *et al.* 2001), *Ocimum basilium* L. (Sudhakaran and Sivasankari 2002) and *Withania somniferum* Dunal (Saritha and Naidu 2007). Though maize is a monoecious crop, but *in vitro* production of only female flowers was recorded. This might be due to the composition of MS medium supplemented by BAP and casein acid hydrolysate. The production of female flower using shoot tip culture may be used for the production of hybrid in maize crop. *In vitro* flowering could offer novel opportunities for studies into the molecular physiology of flowering under control conditions. Further experiment should lead to better understanding of physiological and molecular events underlying the shift from vegetative to flowering stage (Saritha and Naidu 2007).

Table 1. Cultured shoot tip response of maize cultivars in MS medium supplemented with 2 mg/l BAP and 500 mg/l casein hydrolysate after nine weeks.

Variety	No. of shoots developed per shoot tip	No. of leaves developed per shoot tip	Cob induction per shoot tip	Silk production per shoot tip
Vivek-4	6.4	18.8	–	–
Vivek-5	7.8	20.6	13.2	2.3
VL-42	5.6	16.3	8.6	1.5
Him-129	6.1	12.2	5.7	1.1
** CD = 1%	0.27339	0.27328	0.88	0.68

*Data represent the mean of 20 replicates

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