December 2009 Volume 3 Issue 4

Trade Science Inc.

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 3(4), 2009 [261-266]

Effect of physical mutagen (Gamma rays) on differentiation and multiple shoot production from different explants of *Erythrina variegata* L.

T.Shasthree*, M.A.Imran, N.Narsinga Rao, B.Mallaiah

Biotechnology laboratory, Department of Botany, Kakatiya University, Warangal - 506 009, AP, (INDIA)

E-mail : tadurishastri@rediffmail.com

Received: 18th August, 2009 ; Accepted: 28th August, 2009

ABSTRACT

"*In vitro* mutagenesis" is defined as the induction of mutation in cell cultures maintained *in vitro* by the use of chemical and physical mutagens and subsequent establishment of cell lines and regeneration of mutant plants. In the present paper, the effect of gamma rays was studied on morphogenesis, rooting efficiency, caulogenesis and multiple shoot production in *Erythrina variegata* L. Among the explants studied stem and petiole have responded well in induction of caulogenesis, rhyzogenesis and multiple shoots in 3-5 kR gamma irradiation.

KEYWORDS

In vitro mutagenesis;
Gamma rays;
Erythrina variegata;
Multiple shoots;
Caulogenesis;
Rhyzogenesis;
Explants.

INTRODUCTION

Induced mutations (as opposed to naturally occurring mutations) are of great use for plant breeding, either directly to improve specific traits, or indirectly for cross breeding experiments[1]. Cells have evolved an elaborate set of enzymes to counteract the DNA damage. They can repair and maintain DNA integrity, making natural mutations quite rare. The principle of *in vitro* mutagenesis, therefore, is to device a scheme by which we can induce DNA lesions in a certain population of cells maintained *in vitro* and allow these cells to divide rapidly so that the repair mechanism introduces minor errors in the nucleotide sequence of the DNA. As a result, the selected population of cells would have mutations in specific genes, and if whole plants were regenerated from such cells, one would obtain mutant plant lines. The application of *in vitro* mutagenesis has vast potential for increasing the available genetic variants in the years to come. By the year 2000, over 2,200 mutant varieties of plants (mostly ornamentals) had been released world wide (FAO / IAEA Statistics), including 175 crop plant species with induced mutant varieties[2]. The mutagens cause various kinds of DNA damage, such as deletion or duplication of nucleotides, or rearrangements (inversion, translocation) of segments of DNA in the chromosomes. Some of the base pair deletions and substitution (e.g., exactly three bases of a codon within a gene) may not lead to frameshift mutations and may not result in any change of phenotype.

The definition of the term "*In vitro* mutagenesis" mean "induction of mutation in cell cultures maintained *in vitro* by the use of chemical and physical mutagens and subsequent establishment of mutant cell lines and /

or regeneration of mutant plants". Plant tissue culture in conjuction with mutation breeding and related techniques is a promising and potentially emerging areas of plant biotechnology and has generated great interest and speculation for genetic manipulation of crop plants with desirable results. Mutagenesis *in vitro* is an important field for crop improvement[3]. A combination of explant irradiation and *in vitro* regeneration is mostly effective for manifestation of variants[4]. Radiations are potentially useful for introducing plant mutants through tissue culture. The recent *in vitro* mutagenic technology has for reaching application in agriculture and it is envisaged that the next agricultural revolution will primarily be based on *in vitro* technology. Following the discovery that ionizing radiation[5,6] and mutagenic chemicals[7], considerable options led to practicability of the mutagenesis technique for improving productivity of a vast array of organisms useful to man[8].

Several techniques in plant cell culture have been used to isolate a number of mutants in various crop plants which are available at the cultivar level[9,10]. The application of physical mutagens in tissue cultures has been reported by several authors[11]. Ionizing radiation viz., X-rays and gamma rays are most useful in producing plant mutants through tissue culture. There is extensive study on the effects of ionizing radiations and biochemical, physiological and morphological changes in the plant system[12].

There was considerable work on *in vitro* mutagenesis especially followed gamma-irradiation on seeds, seedling and callus culture for their morphological and physiological variations[13]. There were reports that lower doses of gamma irradiation was stimulatory in producing callus[14]. The subject of induced mutations in tissue cultures has been extensively reviewed by several authors[15,16]. Stimulatory effects of low doses of ionizing radiations, not only on growth but also on different in cultured plants cells, was demonstrated by several workers[17]. Kochba and Spiegel Roy demonstrated that by irradiation and addition of certain growth regulators like IAA to the medium the response of *Citrus sinensis* tissue culture was enhanced[18].

There are no reports of *in vitro* mutagenic studies in *Erythrina variegat* L. only a few reports are available in certain plant species of leguminaceae. In *Pachyrrhizus erosus* there are reports on *in vitro* mutagenesis[19]. Ghosh *et al*.[20] studied the effect of gamma rays on callus cultures of *Vigna sinensis*. Mutagenesis *in vitro* can increase variability since adventitious shoot buds develop from simple callus. Bajaj[21] studied the effect of gamma irradiation on growth, RNA, protein and nitrogen contents of bean callus. According to him the growth of the tissue cultures stimulated by low levels of radiation and increasing dosimetry, RNA and soluble protein content continued to decrease. Bajaj *et al*.[22] treated seeds, seedlings and callus tissues of *Phaseolus vulgaris* with gamma rays at 0.5 – 40 kR and found callus tissue was most tolerant than the seedlings and buds. Verma and Van Huystee[23] observed the formation of gaint cells in ground nut cell suspension cultures because of high doses of ionizing radiations. Sham Rao and Narayana Swamy[17] irradiated callus tissue of *Cajanus cajan* with gamma rays at 0-30 kR and found that irradiation helped in plant regeneration.

In the present investigation the effect of gamma rays on morphogenesis, rooting efficiency, caulogenesis and number of shoot production were studied.

RESULTS

Effect of gamma rays

Gamma ray irradiated explants

Petiole explant cultures (Plate I, Figure 1-4)

The petiole explants collected from seedlings were irradiated with 5 kR and 10 kR gamma rays. The explants exposed to 5 kR responded well in callusing on MS medium supplemented with 2.0 mg/l, 2,4-D and 0.5 mg/l BAP. There is a high proliferation of callus in subcultures. The browning of calli were observed in long term cultures. Colour of the callus may be white, yellowish, green and brown. The texture of the callus is either friable or hard compact.

The explants exposed 10 kR gamma irradiation induced multiple shoots (Plate I, Figure 3) on MS + 1.0 mg/l TDZ + 15% coconut milk. Plant regeneration was observed in 5 kR gamma irradiated petiole explants (Plate I, Figure 4).

Stem explant cultures (Plate I, Figure 5-8)

Plate I

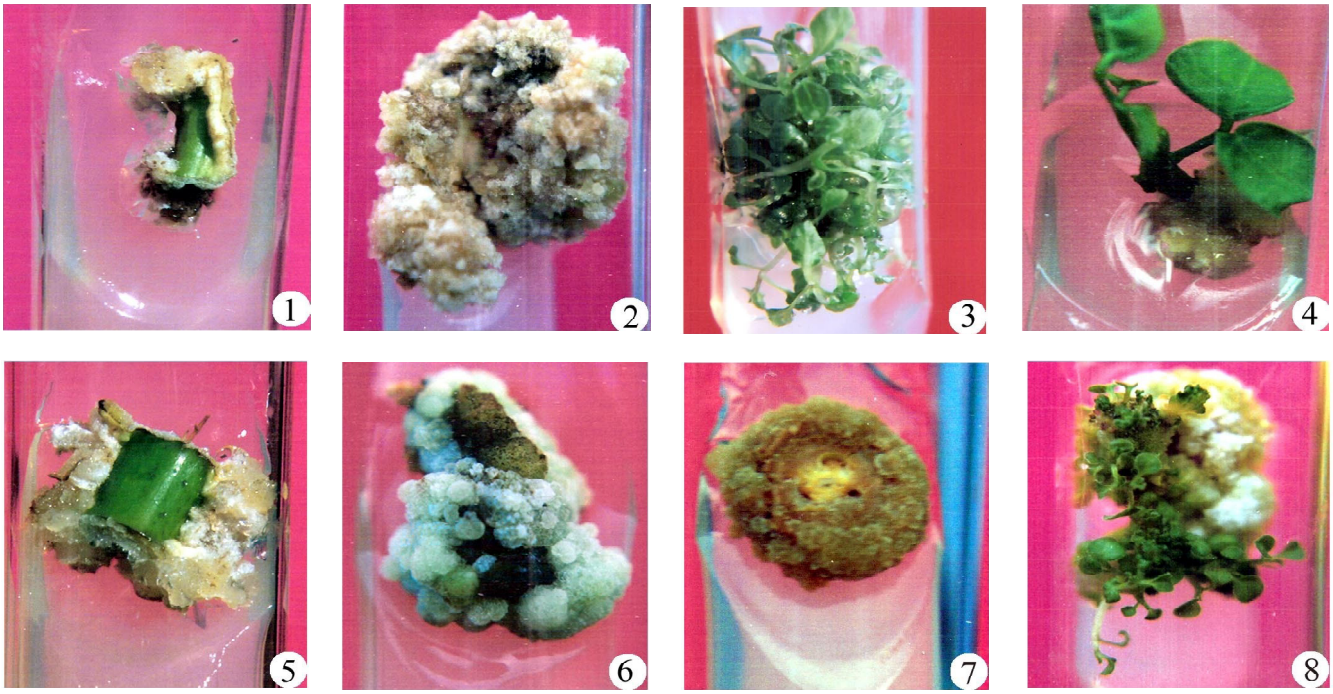
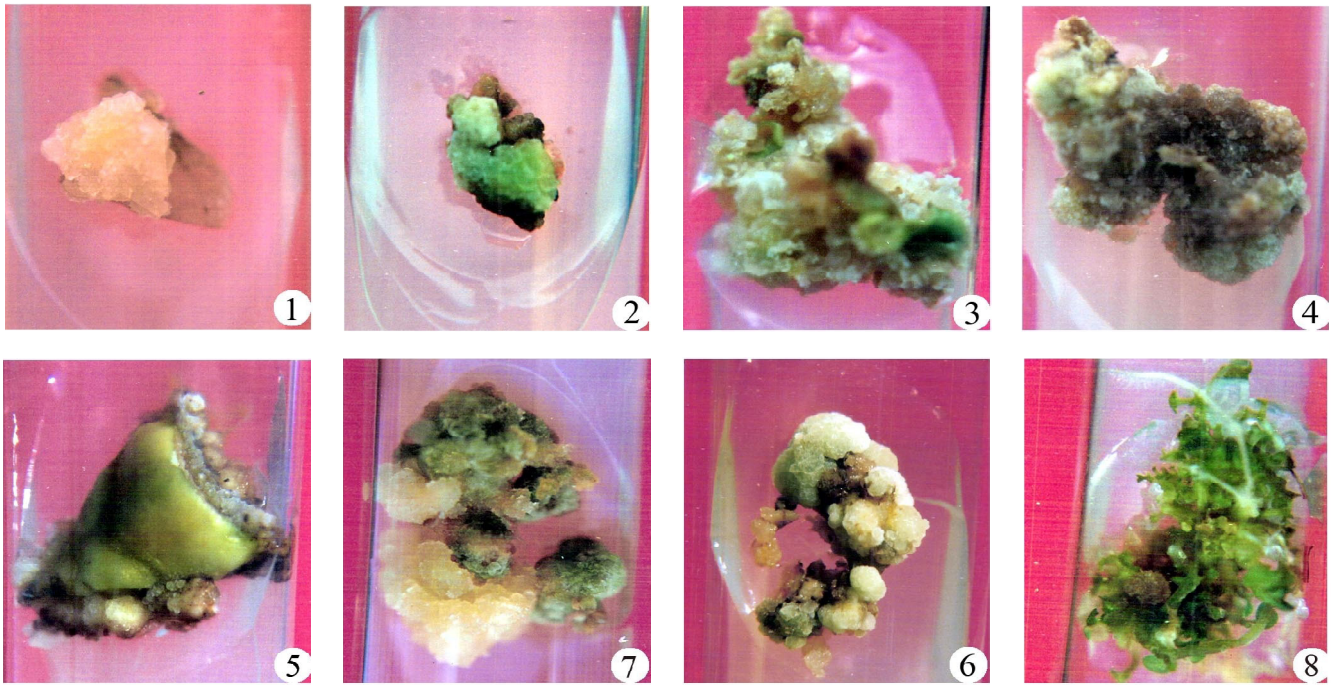


Plate II



During the present studies it was observed that the callusing ability from irradiated stem explants increased from 5 kR dose to 15 kR dose on MS medium with 2.0 mg/l BAP + 1.0 mg/l, 2,4-D + 2.0 mg/l L-glutamic acid (Plate I, Figure 5). The percentage of growth response and morphogenetic response was presented. The maximum percentage of callusing, multiple shoot formation and rhizogenesis was observed on MS + 2.0 mg/l BAP + 1.0 mg/l 2,4-D + 2.0 mg/l L-glutamic acid and it was 85% stem irradiated with 5 kR responded well to this maximum percentage of growth response (Plate I, Figure 8). After subcultures greening of callus and globular like structures appeared on the same me-

FULL PAPER

dium (Plate I, Figures 6 & 7). There was a significant increase in fresh and dry weights of callus in the lower doses whereas at higher doses there was a progressive decrease in the fresh weight and dry weights when compared to control callus. Regeneration of plantlets induced from the callus exposed to 10 kR gamma irradiation (Plate I, Figure 8).

Leaf explant cultures (Plate II, Figure 1-4)

Young leaves cut from seedling explants were exposed to varied doses i.e., from 1 kR to 20 kR. The leaf explants were used for the induction of callus by cutting the leaves into small pieces of about 2-3 sq cm size. The pieces were inoculated on the MS medium supplemented with 2.0 mg/l TDZ + 1.0 mg/l NAA (Plate II, Figure 1). Started proliferation after 15 days of the inoculation on MS + 1.0 mg/l TDZ + 0.5 mg/l NAA. The maximum percentage of cultures with growth (callus response was achieved by increasing the concentration of L-glutamic acid). Above levels of 2. 0 mg/l TDZ reduces the growth response. Green spots appeared on 2.0 mg/l BAP + 1.0 mg/l NAA (Plate II, Figures 2 & 3). There was a significant increase in fresh weights and dry weight with an increase in dose of gamma rays from 1 kR to 10 kR and after there has been gradual decrease.

Cotyledon cultures (Plate II, Figure 5-8)

The irradiated (5 kR) cotyledons of *Erythrina variegata* were inoculated on MS medium supplemented with 2.0 mg/l 2,4-D and 1.0 mg/l BAP. BAP alone could proliferate callus from cut ends but the percentage of cultures showing response were less (Plate II, Figure 5). In 35% of cultures showed the callus proliferation on 0.5 mg/l BAP + 2.0 mg/l L-glutamic acid and 2.0 mg/l 2,4-D. The combination of 1.0 mg/l 2,4-D + 2.0 mg/l L-glutamic acid promoted compact and profused callus formation. On MS medium supplemented with above phytohormones green granular callus proliferation was observed and later the callus turned brown (Plate II, Figure 7). Rhizogenesis was observed on 2.0 mg/l BAP + 1.0 mg/l 2,4-D + 2.0 mg/l L-glutamic acid. The cotyledon callus irradiated with 10 kR doses on MS + 2.0 mg/l BAP + 1.0 mg/l TDZ + 1.0 mg/l L-glutamic acid responded well for plant regeneration.

TABLE 5 : Morphogenetic response of leaf explants derived from gamma irradiated seedlings on ms medium with 2.0 mg/l BAP + 1.0 mg/l NAA and 2.0 mg/l L-glutamic acid.

Dose (kR)	% of cultures with growth response	Morphogenetic response
Control	65	Callus + Shoots + Roots
1	72	Excessive callus
2	78	Shoots and Roots
3	85	Multiple shoots
4	90	Multiple shoots
5	80	Initiation of callus
10	45	Initiation of shoot buds
15	NR	Proliferation of death callus (after 4 weeks)
20	NR	Proliferation of death callus (after 2 weeks)

Data scored at the end of 6 weeks of culture; NR = No response

DISCUSSION

Radiation induced mutations have been extensively used for the improvement of crop plants. A combination of *in vitro* techniques and radiation induced mutagenesis has been recommended to improve plants[2]. Genetic variability can be induced through *in vitro* mutagenesis. Mutagenesis *in vitro* is an important field for crop improvement[3]. A combination of explant irradiation and *in vitro* regeneration is mostly effective for manifestation of variants[4].

In the present investigation *in vitro* mutagenesis was used to study the effect of gamma irradiation on callus induction and organogenesis. Most of the observations and findings have confirmed the earlier reports. The effect of gamma rays in tissue culture has been reported in different plant materials[24,25]. In the present study on the effect of gamma rays on callus induction, the lower doses of irradiation promoted the callus growth and higher doses decreased it. Such findings were reported by Venkateswaran and Partanen[26] in tobacco. George and Rao[27] reported the doses like 2,3 and 4 kRs favoured callus and multiple shoot induction. When excised hypocotyl segments of *Cajanus* seedlings obtained from gamma irradiated seeds were grown on MS medium containing 2,4-D + Kn. Hypocotyl segments was observed. Proliferation of the tissue was more marked in the segments that had received 5 kR[17]. Mustafa *et al.*[28] reported the effect of gamma radiation on morphogenesis from different explants of bitter

gourd *Momordica charantia*. Effect of gamma irradiation on cotyledon cultures of mustard plants were demonstrated by George and Rao[27]. The effect of gamma irradiation on growth and cytology of carrot tissue culture was reported by Bassam, Safadi and Simon[29]. The exposure dose and dose rate of gamma irradiation on shoot forming capacity of cotyledon explants of red pepper was studied. In the present study variations in leaf, floral characters, induction of callus and shoot formation were observed. This finding is supported by Mustafa *et al*.[30]. The response of cells to radiations are said to be dose dependent according to Arya and Hilbdrandt[31] working on grape stem callus. Bajaj[21] used doses of 0, 1, 2, 3, 10, 20, 30 and 40 kR gamma rays on callus cultures of *Phaseolus vulgaris* to study their effect on total protein and RNA.

The morphogenesis was observed in *Erythrina variegata* after two subcultures. King[32] showed that irradiated tobacco callus induced the initiation at meristem in an adjacent non-irradiated fragment. Effect of irradiated sucrose on morphogenesis was also studied[33]. It was suggested that radiation induced organogenesis results from inactivation of auxin by radiation, removing the inhibition of bud formation.

The effect of gamma rays was studied on multiple shoots from different explants i.e., nodal, cotyledon, shoot tip, hypocotyl and stem of *Erythrina variegata*. Among all the explants used, nodal explants were the best for mltiple shoot induction followed by shoot tip, cotyledon, hypocotyl and stem explants. Development of more number of shoots from shoot-tip than from the nodal and cotyledons indicates that the former explants have a high regenerative capacity, which promoted regeneration of shoots and inhibited regeneration of roots in the presence of cytokinins.

During the studies it was observed that lower concentration of BAP and L-glutamic acid promoted the multiple shoot induction in almost all the explants studied. BAP was found essential for shoot multiplication. Other leguminous tree species where BAP-induced shoot multiplication has been reported are *Acaci*[34], *Dalbergia*[35], *Albizzia*[36] and *Leucaena*[37]. In *Prosopis*, however, Kn with IAA proved better than BAP[38]. BAP and glutamine promoted shoot multiplication in *Pterocarpus santalinus* in the second passage but thereafter the rate of shoot proliferation declined. This may probably be due to the endogenous levels of certain factor/s, inherited with the explants, which is / are gradually diluted with each passage and is / are completely lost after the third passage. The combination of cytokinins and auxins stimulate the *in vitro* multiplication and the growth of shoots of several plant species[39]. However, in our case, the addition of either NAA, IAA or IBA to the multiplication medium (MS with BAP) significantly reduced the number of shoots per explant and did not affect shoot length. Lower concentration of auxin (NAA) and cytokinins (BAP) induced callus from nodal and cotyledon explants. Such results were supported by earlier findings. Halder and Galdgil[40] reported the shoot bud differentiation in *Momordica* and *Cucumis*. The frequency of adventitious buds were increased with increase in concentration of BAP or TDZ after irradiating the cotyledons in *Cucumis sativus*[41]. During the course of investigation the higher concentrations of BAP (3.0 mg/l) induced multiple shoots in all explants studied TDZ proved the most effective cytokinin in inducing shoot bud formation, which resembles the earlier reports of *Capsicum*[42]. Multiple shoot buds were differentiated from cotyledon explants or *Mathioloa incana* cultured on BAP or TDZ[43]. TDZ and BAP combinations were recorded to induce direct shoots in the present investigation similar observations were made in *Brassica* spp.[44] and Castor[45]. TDZ, BAP combinations were provided superior to BAP-Kn combination in inducing shoots in tomato[46].

REFERENCES

[1] Negrutiu; 'Procedures and Applications', P.J.Dix (Ed.), Weinheim, Germany: VCH Publishers, 19-38 **(1990)**.
[2] M.Maluszynski, B.S.Ahloowalia, B.Sigurbjhornsson; Euphytica., **85**, 303-315 **(1995)**.
[3] P.J.King; Acad., Press, Orlando, 547-551 **(1984)**.
[4] F.J.Novak, A.Micke; Sabrao., **19**, 82-85 **(1987)**.
[5] H.J.Muller; Science., **46**, 84-87 **(1927)**.
[6] L.J.Stadler; Science., **68**, 186-187 **(1928)**.
[7] C.Auerebach; Biol.Rev., **24**, 355-391 **(1949)**.
[8] V.L.Chopra, R.P.Sharma; Oxford and IBH publishing Co., New Delhi, **(1985)**.
[9] W.Handro; Plant Cell Reports., **2**, 133-136 **(1983)**.
[10] M.Lee, R.L.Phillips; Genome., **29**, 122-128 **(1986)**.

FULL PAPER

[11] R.M.Skirvin; Euphytica., **27**, 241-266 **(1978)**.
[12] J.E.Gunkel, A.H.Sparrow; Encyclopedia of plant physiology., **16**, 555-611 **(1961)**.
[13] V.A.Bapat, P.S.Rao; Plant Sci.Lett., **6**, 291-298 **(1976)**.
[14] D.R.Sharma, S.Dawra, J.B.Choudhary; Curr.Sci., **52**, 606-607 **(1983)**.
[15] H.E.Street; Plenum Press, New York, 231-244 **(1974)**.
[16] P.J.Bottino; Rad.Bot., **15**, 1-16 **(1975)**.
[17] H.K.Sham Rao, Narayanaswamy; Mill sp.Rad.Bot., **15**, 301-305 **(1975)**.
[18] J.Kochba, P.Spiegel-Roy; Pl.Br.Abs., **48**, 2 **(1978)**.
[19] Satyanarayana Murthy; Ph.D.dissertation, Kakatiya University, Warangal, **(1999)**.
[20] Ghosh, G.C.Mitra, A.K.Sharma; Indian Bot.Reptr., **70**, **(1984)**.
[21] Y.P.S.Bajaj; Ann.Bot., **34**, 1084-1096 **(1970)**.
[22] Y.P.S.Bajaj, A.W.Satteler, M.W.Adams; Rad.Bot., **10**, 119-124 **(1970)**.
[23] D.P.S.Verma, R.B.Van Huystee; Rad.Res., **48**, 518-530 **(1971)**.
[24] N.Degani, D.Pickholz; Rad.Bot., **15**, 363-366 **(1973)**.
[25] P.A.Werry, J.Th, K.M.Stoffelsen; Theor.Appl. Genet., **59**, 391 **(1981)**.
[26] S.Venkateshwaran, C.R.Partanen; Rad.Bot., **6**, 13-20 **(1966)**.
[27] L.George, P.S.Rao; Ann.Bot., **46**, 107-112 **(1980)**.
[28] M.D.Mustafa, B.Noel Nirmala, A.M.Rao, B.Mallaiah; Nat.Acad.Sci.Letters., **16(3)**, 101-102 **(1993)**.
[29] A.L.Safadi Bassam, W.Simon Phillip; Envir.and Exptl.Bot., **30(3)**, 361-371 **(1990)**.
[30] M.D.Mustafa, M.Parthasarathy, B.Mallaiah; Ad.Plant Sci., **4(2)**, 419-422 **(1991)**.
[31] H.C.Arya, A.C.Hilbrandt; Can.J.Bot., **47**, 1623-1628 **(1969)**.
[32] C.S.King; Am.J.Bot., **36**, 265-270 **(1949)**.
[33] P.V.Amirato, F.C.Steward; Develop.Bio., **19**, 87-106 **(1969)**.
[34] R.G.Skolmen, M.O.Mapes; J.Hered., **67**, 114-115 **(1976)**.
[35] A.Mukhopadhyay, H.Y.Mohan Ram; Indian J.Exp.Biol., **19**, 1113-1115 **(1981)**.
[36] S.Upadhyaya, N.Chandra; Ann.Bot., **52**, 421-424 **(1983)**.
[37] V.Dhawan, S.S.Bhojwani; Plant Cell Reports., **4**, 315-318 **(1985)**.
[38] Y.Goyal, H.C.Arya; J.Plant Physiol., **115**, 183-189 **(1984)**.
[39] E.F.George; The Technology Exegetics Ltd., Edington, **(1993)**.
[40] T.E.Halder, V.N.Gadgil; Ind.J.Exptl.Biol., **20**, 780-782 **(1982)**.
[41] M.D.Mustafa, B.Mallaiah; J.Mendel., **8(3)**, 141-148 **(1991)**.
[42] P.Sripichitt, E.Nawata, S.Shigenaga; Japan J.Breed., **17**, 133-142 **(1987)**.
[43] V.K.Gautaum, A.Mittal, K.Nanda, S.C.Gupta; Plant Sci.Lett., **29**, 25-32 **(1983)**.
[44] S.B.Narasimhulu, V.L.Chopra; Plant Cell Report., **7**, 104-106 **(1988)**.
[45] K.R.K.Reddy, G.P.Rao, Birbahaddur; Phytomorphology., **37**, 337-340 **(1987)**.
[46] K.K.Kartha, O.L.Gamborg, J.P.Shyluk, F.Constable; Z.Pflanzen.Physiol., **77**, 292-301 **(1976)**.