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Research Article

AN EFFICIENT *IN VITRO* REGENERATION PROTOCOL FOR AN ENDANGERED MEDICINALLY IMPORTANT HERB *FAGOPYRUM DIBOTRYS* GROWING IN KASHMIR HIMALAYA

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ABSTRACT
The establishment of a simple and efficient protocol for shoot organogenesis and regeneration from nodal explants of Buckwheat *Fagopyrum dibotrys* during the present investigation has been reported.The effect of different concentrations of cytokinins (BAP) and Auxins on the efficiency of shoot organogenesis in buckwheat was confirmed. Treatment with BAP significantly induced shoot regeneration from nodal cuttingsand the best results were achieved in MS basal medium supplemented with $2mgl^{-1}$BAP + $1mgl^{-1}$IBA. However the highest number of shoots per explant (3.8) and shoot length (3.36cm) were obtained on MS medium containing $3.0mgl^{-1}$BAP. The best medium for induction of roots in the micro shoots of was MS medium adjuvanted with $2.5mgl^{-1}$IBA.The rooted plants were hardened and transferred to soil with a 72% survival rate.

Keywords: *Fagopyrum, Micropropagation, Nodal Cuttings, Benzyl Amino Purine, Indole Butyric Acid*

INTRODUCTION
Fagopyrum dibotrys belongs to genus *Fagopyrum* (Polygonaceae) and is native to temperate East Asia. It prefers to grow in the areas where soil moisture is high. It has been included in the National Register of Highly Protected Wild Plants. Its seeds contain 18 varieties of amino acid of which 8 varieties are essential to human body. The content of amino acids is higher than those in principal buckwheat cultivars and is much higher than those in rice, wheat and maize. It contains abundant bioflavonoids and the major components of bioflavonoids are quercetin, rutin, morin and others, which serve well to dilate blood vessels and to reduce cholesterol level (Ma-Rong 2001). The whole plant is anodyne, anthelmintic, antiphlogistic, carminative, depurative and febrifuge. It has markedly anticancer effects. The growth of cancer cells from lung, liver, colon, leukocytes and bone is inhibited by *Fagopyrum dibotrys* (Zhong, 2003).

Medicinal and Economic Importance
In India, along with North Eastern States, the crop is widely grown in Jammu and Kashmir (Joshi, 1999). Five species of Fagopyrum viz. *Fagopyrum esculentum*, *Fagopyrum tataricum*, *Fagopyrum saggittatum*, *Fagopyrum dibotyrs* and *Fagopyrum kashmirianum* are found in the Kashmir Himalaya and Ladakh region. It is a quick growing crop. It grows on the worst and poorest soils. It prefers a moist cool climate and a well drained sand soil.

It is sown in July and harvested in October. It is used in house hold remedies. Leaves are cooked in iron vesselare given to anemic patients. This cooked leaves are also used to cure old constipation (Pant *et al.*, 2009). It is used as a good source of dietary Rutin (Koda *et al.*, 2008; Nassiri-Asl *et al.*, 2008; Yang *et al.*, 2008) because no Rutin was found in cereals and pseudo cereals accept this species (Kreft *et al.*, 2006).

The presence of Rutin content was reported in the processed groats, leaves, and flowers of buckwheat (Park *et al.*, 2000). Local people in hilly area of Ladakh used it in cooking and on the occasion of festivals, and other religious rituals, dishes are prepared out of it (Mehta *et al.*, 2010).

The protein of buckwheat is of excellent quality and is high in the essential amino acid lysine, unlike common cereals. Common buckwheat contains high nutritive substances (63% carbohydrate, 11.7% protein, 2.4% fat, 9.9% fibre, 11% water and 2% minerals). Owing to its medicinal importance the plant species has been subjected to *invitro* studies so as to develop an efficient protocol for its large scale propagation.

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MATERIALS AND METHODS

Nodal cuttings of 2-3 cm in length were excised from mature plants. These explants were surface sterilized with 0.1% $HgCl_2$ for four minutes. The pH of the medium was adjusted at 5.8.The media were autoclaved at 121 degree centigrade and 15 psi pressure. The optimum photoperiod for culture and growth of shoots is 16 hours light and 8 hours darkness in a 24 hour cycle.

RESULTS AND DISCUSSION

Results

The surface sterilised nodal cuttings were inoculated on MS basal medium supplemented with BAP at concentrations of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0mgl^{-1}. The sprouting of nodal explants increased with the increase in concentration of BAP and optimum concentration was $3mgl^{-1}$(Table No. 1). At this concentration an average number of 3.8 shoots/node were produced in about 100% of cultures. With further increase in concentration of BAP the average number of shoots decreased.

Table 1

M S +BAP mgl^{-1}	**Mean number of shoots per explant**	**Average length of shoots (cm)**	**%age cultures**
0.5	No Response	No Response	No Response
1.0	1.0	1.04	100
1.5	1.6	1.7	100
2.0	1.8	2.0	100
2.5	2.4	2.8	100
3.0	3.8	3.36	100
3.5	2.0	2.18	100
4.0	1.2	1.52	100

(Ten replicates for each experiment)

Standardization of Media for in Vitro Rhizogenesis

Microshoots from the proliferated cultures were aseptically excised and transferred to MS medium supplemented with various concentrations of auxins (IBA, NAA). The growth regulators were used individually as well as in combination. The MS medium was used with full strengthsalt concentrations.

(A) Sprouting of nodal cuttings

(B) Induction of roots

Table 2: Effect of IBA and IAA supplemented MS basal medium on root induction

MS+ IBA mgl^{-1}	**Mean number of roots per micro shoot**	**Average root Length(cm)**
0.5	**-**	
1.0	**1.4**	**1.94**
1.5	**2.0**	**2.44**
2.0	**2.6**	**2.96**
2.5	**4.6**	**5.2**
3.0	**3.6**	**4.02**
3.5	**2.8**	**3.6**
4.0	**1.6**	**2.96**
MS+IAAmgl^{-1}		
0.5	**-**	**-**
1.0	**0.8**	**1.8**
1.5	**1.4**	**2.4**
2.0	**3.6**	**3.7**
2.5	**2.0**	**2.8**
3.0	**1.4**	**1.38**
3.5	**1.2**	**0.72**
4.0	**0.6**	**0.44**

Discussion

All nodal explants grown on media supplemented with various BAP concentrations survived after four weeks of culture. The bud breaking was slightly increased with 1.0mgl^{-1} BAP followed by 1.5mgl^{-1} BAP; 2.0mgl^{-1} BAP and 2.5mgl^{-1} BAP. Maximum proliferation of shoots was obtained at MS+ 3.0mgl^{-1} BAP with mean number of 3.8 shots from each explant with mean shoot length of 3.4cm [Table No.1; Figure (A)]. A concentration of greater than3.0mgl^{-1} BAP showed apparent decrease in number of shoots. After the successful production of shoots, the *in vitro* raised shoots were subjected to subculturing for root induction. MS basal media supplemented with IBA and IAA of different concentrations was tested for root induction.

However MS+IBA 2.5$mgl^{-1}$and MS+IAA 2.0mgl^{-1}showed better results [Table No. 2; Figure (B)]. Therefore it was concluded that these concentrations could be the optimal concentrations for the successful induction of roots thereby leading to the complete regeneration of plantlet. Also these regenerated plants could be used in a possible transformation protocol which might create new opportunities to study the molecular and metabolic regulation of producing useful secondary metabolites in *F. dibotrys*. The optimum temperature and relative humidity maintained for the culture was 25$\pm$2°C and 70 – 75% respectively. The findings suggest that efficient plant regeneration of *F. dibotrys* via shoot organogenesis was achieved, and that stable regenerated normal shoots were also successfully obtained from adventitious bud regenerants.

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