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Review Article

Plant Regeneration and Genetic Transformation in Buckwheat (*Fagopyrum* spp.), A Multipurpose Gluten Free Crop of High Nutraceutical Importance: A Critical Review

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Abstract: Buckwheat (*Fagopyrum* sp.) from family Polygonaceae, with its two main cultivated species (*F. esculentum*Moench. and F. *tataricum*) is mostly grown in hilly regions of Eurasia. It is a multipurpose gluten free crop having great nutraceutical value. For plant propagation several reports have been published and significant differences were noticed in culture protocols, genotypes and their success rates. A few studies report genetic transformation in buckwheat with varying degree of success. The present study reviews the *in-vitro* regeneration and genetic transformation reports in *Fagopyrum* spp. available in publically available literature and concludes on what needs to be done for the sustainable genetic improvement of buckwheat. A genotype independent high frequency *in-vitro* regeneration protocol compatible with high end stable genetic transformation is the need of the hour for sustained genetic improvement of this crop.

Keywords: *Agrobacterium rhizogenes, A. tumefaciens,* Buckwheat, *Fagopyrum dibotrys, F. esculentum*, *F. tataricum*, Genetic transformation, Hairy root culture, *In-vitro* regeneration, Organogenesis, Rutin, Somatic embryogenesis.

Introduction

Buckwheat belongs to genus *Fagopyrum* of family Polygonaceae and it includes 15 annual and perennial species mainly occurring in highlands of Euro-Asia (Arora and Engels, 1992; Ohnishi, 1995). Two main cultivated species are *Fagopyrum esculentum* (common buckwheat) and *Fagopyrumtataricum* (tartary buckwheat) (Chauhan *et al.,* 2010). Although its name mentions 'wheat' in it, buckwheat is not taxonomically related to wheat and it has been accepted as a pseudo cereal crop (Joshi and Rana, 1995). The crop grows well in moist and cool climate with very little nutrient demand and is prevalently cultivated in northern hemisphere. As it can grow in rather poor soils and does not require extensive fertilization, its products are deemed to be a low-cost supplement to cereal grains. Interestingly, however, FAO states show a sharp decline in world area which harvested common buckwheat (from 4.9 MHa in 1992 to 2.01 MHa in 2014). Hailed to be a multipurpose crop, buckwheat is used for both grains and greens such as food, feed, medicine and manure (Dutta *et al.,* 2010). For human consumption, this crop is generally used as vegetable crop, honey crop and smother crop (Campbell, 1997), and however it has significant nutraceutical importance as well. The seeds of buckwheat are used to make bread, pancake, noodles and other food (Kim *et al.,* 2010). Buckwheat is one of the most important gluten free crops and its seeds contain high amount (10-12.5%) of protein (Greenway and Munns, 1980), large amount of lysine and a well-balanced content of other essential amino acids (Bratic *et al.,* 2007). At present, the demand of buckwheat is due to its excellent properties- nutritional values based on the favorable composition of protein complexes with high content of lysine, fibrous material, mineral compounds, vitamins (thus making it quite complementary to cereal flours) and medicinal value due to presence of flavones, flavonoids, sterols, fagopyrin, and thiamin-binding proteins in buckwheat seeds and other parts thus possessing potential effects in treating some chronic diseases (Kreft *et al.,* 2006). Out of all the bioflavonoids that buckwheat contains, the chief component (80%) is rutin, which is a flavonol glycoside. The presence of rutin was reported in the processed groats, leaves, and flowers of buckwheat (Park *et al.,* 2000). Rutin is a good dietary supplement and apart from having several medicinal properties like-vasconstrictive, spasmolitic, anti- inflammatory, anti-carcinogenic, anti-thrombotic, cytoprotective, it is also known to have very good anti-oxidant and anti-hypertensive properties. The seeds of tartary buckwheat contain higher amounts of rutin (about 0.8-1.7% d.w.) than those of common buckwheat (0.01% d.w.) (Fabjan *et al.,* 2003).

Despite all unique potential, buckwheat never achieved the status of important crop for cultivation and has never been widely adopted. Traditional breeding for improvement of this crop has been scarce. *Fagopyrum* spp. are diploid, 2n =16; but tetraploid varieties either occur spontaneously or can be induced (Chauhan *et al.,* 2010). Obstacles behind the limited conventional breeding are its strong self/cross-incompatibility, low seed set, indeterminate type of growth and flowering and its susceptibility to spring and fall frosts. The causes of low seed set are unknown but have been attributed to high temperature, plant water stress, plant age, incompatibility caused by heterostylism, defective reproductive organs, failure of fertilization and embryo and/or endosperm abortion (Slawinska *et al.,* 2009). Poor germplasm collection, databases and scarce availability of wild relatives further limit the progress of varietal improvement. Vegetative propagation through cuttings or other conventional methods has not been successful in buckwheat. However, some efforts have been made to study *in-vitro* regeneration in *Fagopyrum* involving different types of approaches and starting material. Yamane was the first to demonstrate *in-vitro* regeneration in common buckwheat as early as 1974. Afterwards, during last four decades regeneration in buckwheat through tissue culture

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has attracted many researchers. Suvorova (2016) reviewed buckwheat tissue cultures and genetic transformation. The author opined that buckwheat regeneration evolved in many different ways and regenerants may be obtained from various explants utilizing different approaches and that tissue culture systems of buckwheat are available for use in both basic and applied research. We in the present work have however identified the gaps in the studies and endeavor to conclude on what needs to be done for improvement of tissue culture and transformation standardizations in this plant species. Buckwheat tissue culture system has very recently been utilized to gain understanding of the mechanisms that are responsible for the genetic stability of cells in the culture. Betekhtin *et al.,* 2017 has reported such a study in tartary buckwheat. They analyzed the histological sections of morphogenic and non-morphogenic callus lines by transmission electron microscopy and described the morphology of the nuclei in these lines. The results showed that non-morphogenic calli cells had aneuploidy, whereas greater genomic stability was identified in morphogenic callus lines.

Buckwheat is a salt-sensitive glycophyte and soil salinity significantly affects the growth and grain yields of its seeds (Chen *et al.,* 2008). Thus, genetic improvement of buckwheat with higher salt tolerance and for other biotic and/or abiotic stresses would produce remarkable economic and medicinal benefits. Nothing very significant, however, has been done by buckwheat breeding in this context. For crop improvement, plant breeding has been a successful and time-tested approach to develop new varieties and hybrids. But by enlarge the approach can utilize the gene pool of the same plant species or its wild relatives to have any activity thereupon. The plant genetic engineering approach, in contrast, can pave the way to assess much wider gene pool across kingdoms and to incorporate agronomic and quality traits not present in any of the crop germplasm or its wild relatives. Although reports with high transformation efficiency is lacking, researchers find increasing interest in genetic transformation of buckwheat to incorporate some useful genes governing quality characters or to increase rutin content in buckwheat through hairy root culture. The present study reviews *in-vitro* regeneration reports and the methods, approaches, physical and nutritional requirements and success rates contained therein on *Fagopyrum* as well as reports on genetic transformation in *Fagopyrum* and the efficiency of the processes.

Plant Regeneration in *Fagopyrum*: Materials, Basal media and Media addenda and Incubation conditions

Plant Material: Plant tissue culture of *Fagopyrum* employs different parts of the plant body. Explants from either seedling or differentiated plant parts from mature plant body have been used. Yamane (1974), however, used mature seeds as explants, without any excision, whereas Neskovic *et al.,* 1987 excised immature zygotic embryos and induced somatic embryogenesis in buckwheat. In all the previously published reports, the most widely used explants have been hypocotyl segments (Adachi, 1989; Lachmann and Adachi, 1990; Gumerova *et al.,* 2001, 2003; Jin *et al.,* 2000; Han *et al.,* 2011; Kwon *et al.,* 2013; Hou *et al.,* 2014) and cotyledon pieces (Srejovik and Neskovic, 1981; Luthar and Marchetti, 1994; Woo *et al.,* 2000; Lee *et al.,* 2009) from axenically grown seedlings. Some reports have utilized both cotyledon and hypocotyl explants (Berbec *et al.,* 1999; Klcova and Gubisova, 2008). The seedling explants, wherever have been used were not older than 14 days; mostly 5-7 days old germinated seedlings have been used for explants preparation. Explants from mature plants like leaf (Rajbhandari *et al.,* 1995; Park *et al.,* 1999; Woo *et al.,* 2004), nodes (Chen *et al.,* 2012; Majid *et al.,* 2015) and petiole (Slawinska, 2009) have been used for plant regeneration in buckwheat. Anthers have been used for haploid production in buckwheat (Bohanec *et al.,* 1993). Takahata in 1988 showed regeneration from inflorescence in *F. esculentum*and *F. cymosum*. Experimental materials used were either from diploid or tetraploid plants of buckwheat. Whereas immature zygotic embryos, cotyledon and hypocotyl explants were used for inducing somatic embryogenesis, explants from mature plant parts were employed for organogenesis mode of regeneration.

Sterilization of plant material: Explants isolation is preceded by surface sterilization. Various methods of sterilization were applied. In cases where seed or a seedling part have been used as explants, mostly seeds have been surface sterilized and surface sterilized seeds have been incubated for germination. Adachi *et al.,* 1989, however, exposed dehulled seeds to chlorine gas and washed the seeds with sterile water before keeping the seeds for germination, whereas Berbec and Doroszewska (1999) surface sterilized the dehulled seeds with 10% aqueous solution of hydrogen superoxide and subjected for germination. Wherever the mature seeds have been subjected to surface sterilization, mostly the first step is washing with 70% ethanol, followed by treatment with sodium/calcium hypochlorite, or mercuric chloride or other disinfectants with varying concentration and time and then washing with sterile water to remove traces of disinfectants. (Gumerova *et al.,* 2003; Lee *et al.,* 2009; Han *et al.,* 2011; Chen *et al.,* 2012; Kwon *et al.,* 2013). Disinfectors were usually supplemented with Tween surfactant. Seed were decapped and imbibed in autoclaved distilled water before placing for germination. Surface sterilized seeds or dehulled seeds after surface sterilization were germinated mostly on MS basal medium solidified with a gelling agent (Park *et al.,* 1999; Jin *et al.,* 2002; Klcova and Gubisova, 2008; Lee *et al.,* 2009; Kwon *et al.,* 2013; Hou *et al.,* 2014). Some workers have, however, also utilized half strength MS basal salts along with varying concentrations of sucrose solidified with a gelling agent to germinate buckwheat seeds (Lachmann and Adachi, 1990; Berbec and Doroszewska, 1999; Kachonpadungkitti *et al.,* 2001). On the other hand, Adachi *et al.,* 1989 used simple agar plates to germinate the seeds and Woo *et al.,* 2000 utilized sterile filter paper moistened with water to germinate the seeds.

Basal Media and Media Addenda: Experiments carried out on *Fagopyrum* for *in-vitro* regeneration mostly used Murashige and Skoog (1962) medium as basal medium and supplemented this medium with different plant growth regulators or other addenda. Other media formulations like Gamborg-B_5 (1968), White (1954), RM-1964 and Linsmaier and Skoog (1965) were also employed. Yamane, 1974 used White's basal medium and RM-1964 medium. Srejovik and Neskovic, 1981; Neskovic *et al.,* 1987; Yui and Yoshida, 2001; and Gumerova *et al.,* 2003, however, utilized Gamborg- B5 as basal medium. Berbec and Doroszewska, 1999 utilized Linsmaier and Skoog medium (1965) as basal medium. Different types of plant growth regulators were used as

media addenda- Auxins 2, 4-D and NAA have been frequently used to induce either callus or regeneration and IAA and IBA have been used to induce roots. Whereas cytokinins BAP and Kinetin have been widely used for regeneration in *Fagopyrum*, TDZ and 2iP have also been used. Jin *et al.,* 2002, however, utilized a more complex media by supplementing casein hydrolysate and yeast extract also in MS medium. Neskovic *et al.,* 1987 also utilized 2g/L casein hydrolysate for pretreatment of explants for embryogenic callus induction.

Carbon Source: Sucrose has been mostly used as carbon source. Bohanec, 1993, however, showed that maltose can also be utilized as carbon source for *Fagopyrum* regeneration.

Gelling agents: Most of the reports utilized agar as gelling agent in various concentrations (0.6- 0.8%, w/v). Klcova and Gubisova, 2008 screened three different types of gelling agent treatments- agar, Phytagel and agar along with Phytagel and came out with conclusion that 0.8% agar was the best suited gelling agent. Lee *et al.,* 2009, however, have got good results with 0.3% Gelrite.

Culture conditions: *In vitro* cultures were usually maintained at temperature of 24±3ºC and photoperiod of 16 hours under white fluorescent light in most of the reports. Berbec and Doroszewska (1999) have utilized a photoperiod of 12 hours to incubate the cultures at a temperature of 27ºC. Kachonpadungkitti *et al.,* 2001, however, incubated the cultures at 27±2ºC and under 8 hour's photoperiod. Park *et al.,* 1999 used a lower temperature (22ºC) to incubate buckwheat *in-vitro* cultures.

A comparative and summarized view of explants used, regeneration modes, culture media, plant growth regulators and other media addenda and frequency of *in-vitro* regeneration in *Fagopyrum esculentum* Moench. is presented in Table 1.1 and that of other species of *Fagopyrum* is presented in Table 1.2.

Table 1.1: Explants, regeneration modes, media, plant growth regulators and other media addenda, and frequency of *in-vitro* regeneration in *F. esculentum* as reported in different studies

Explant	Mode	Basal Media	Plant Growth Regulators	Other addenda	Frequency	Reference
Immature zygotic embryo	Somatic embryogenesis	B_5 Medium	2,4-D (1-10mg/L), KIN (0.1 mg/L), BAP(2.2 mg/L), IAA (0.17 mg/L), IBA (0.5 mg/L)	Casein hydrolysate	37.1%	Neskovic *et al.,* 1987
Leaf and Stem	Somatic embryogenesis	MS medium	2,4-D (1 mg/L, 3 mg/L)	--	1.6% - 5.6%	Park *et al.,* 1999
Cotyledon	Somatic embryogenesis	MS medium	2,4-D (1-3 mg/L), Kin(0.2mg/L), BAP (0.5-5 mg/L), NAA (0.1-0.5 mg/L)	--	32%	Woo *et al.,* 2000
Hypocotyl	Somatic embryogenesis	B5 Medium	2,4-D (2-15 mg/L), BA (2.23 mg/L), IAA (0.175mg/L)	--	95%	Gumerova *et al.,* 2001,2003
Hypocotyl	Somatic embryogenesis	MS Medium	2,4-D (1 - 8 mg/L), KIN (1 mg/L), BAP (0.1 - 5mg/L), IAA(2 mg/L)	--	5% - 20%	Kwon *et al.,* 2013
Seed	Organogenesis	White medium, RM-1964 medium	2,4-D (1-10 mg/L), KIN (0.2 - 1 mg/L), IAA(1 -5 mg/L)	Ascorbic acid, casamino acid, coconut milk, yeast extract.	73.3%	Yamane, 1974
Cotyledon	Organogenesis	B5 Medium	2,4-D (1.0,5.0mg/L), KIN (0.1,1.0mg/L), IAA (1.75 mg/L), BAP (22.5 mg/L), IBA(1.0mg/L)	Casein hydrolysate	40%	Srejovic and Neskovic, 1981
Cotyledon	Organogenesis	MS Medium+ B_5 Vits.	IAA(0.5 mg/L), IBA(0.25 mg/L)	--	4.37%	Luther and Marchetti, 1994
Cotyledon and hypocotyl	Organogenesis	LS medium	TDZ (0.01-0.1mg/L), IAA (0.5mg/L), BAP (0.5,0.7mg/L), ABA (0.2 mg/L), 2,4-D (3, 5 mg/l)	--	30% - 50%	Berbec and Doroszewska, 1999
Hypocotyl	Organogenesis	MS Medium	2,4-D(1-2 mg/L), BAP(1-2 mg/L), IBA(0.2mg/L), NAA(0.2 mg/L, 0.4 mg/L), KIN (1mg/L, 2 mg/L)	Casein hydrolysate	80%	Jin *et al.,* 2002
Cotyledon (Co) & Hypocotyl (Hy); Nodal segment (No) and Shoot Apex (SA)	Indirect Organogenesis; Direct Organogenesis	MS medium, B_5 Medium, MS salts with B5 vitamins	BA(0.5 -4.0 mg/L), IAA(0.2 mg/L), IBA(0.2 -2.0 mg/L)	--	(Co) & (Hy) : 1.25-2.44 Shoots/explant (No): 4.1– 4.8 new nodes/ explant SA: 2.65 to 3.33 nodes/ explant	Klcova and Gubisova , 2008
Cotyledon	Organogenesis	MS medium	BAP(0.5-6 mg/L), KIN(0.5 -6 mg/L), TDZ (0.5 -6 mg/L)	$AgNO_3$	72%	Lee, 2009
Hypocotyl	Organogenesis	MS medium	2,4-D(1 – 4 mg/L), BAP(0–2 mg/L), NAA(0.5 -1.5 mg/L)	--	60%	Hou *et al.,* 2015
Leaf	Organogenesis	MS medium	KIN (0.2 mg/L, 0.5mg/L), 2,4-D (2 mg/L), BAP (2 mg//l, 3 mg/L), IAA (various conc.), NAA (0.2 mg/L)	--	30%	Woo *et al.,* 2004
Leaf petiole	Organogenesis	MS medium + B_5 Vits.	BA, 2iP, TIBA at 1 mg/L, 2 mg/L each)	--	98%	Slawinska *et al.,* 2009
Nodal segment	Organogenesis	MS medium	IBA(0.01–20 mg/L), NAA (0.01 –20 mg/L), BAP(0.01 - 20 mg/L), GA (0.01–10 mg/L), KIN (0.01 -20 mg/L)	--	100 %, 10 bloomed flowers / explant	Kachonpadungkitti *et al.,* 2001
Immature Inflorescence	Organogenesis	MS medium, B5 Medium	NAA (0-5 mg/L), BA (0-2 mg/L), IBA (1 mg/L), 2,4-D (0-5 mg/L)	--	40% - 47.6%	Takahata, 1988
Anther	Organogenesis	B5 Medium	NAA(1 -2mg/l), BA(0.5 -2.5 mg/l)	--	43 .3%	Yui and Yoshida, 2001
Anther	Haploid Production	MS medium	2-iP (5 mg/L), 2,4-D (2 mg/L,4 mg/L), BAP(1 -2.5mg/L), IBA (0.5 mg/L), IAA (0.1 -1 mg/L), KIN (2 mg/L)	coconut milk	1.36 %	Bohanec *et al.,* 1993
Hypocotyl Protoplast	Organogenesis	MS Medium	2,4-D (0.1 -1 mg/L), BAP (0.1 -2 mg/L), NAA (0.1 mg/L, 2 mg/L), GA_3 (0.1 mg/L)	--	Not mentioned	Adachi *et al.,* 1989

Table 1.2: Explants, regeneration modes, media, plant growth regulators and other media addenda, and frequency of *in-vitro* regeneration in *F. tataricum, F. dibotrys* and *F. cymosum* as reported in different studies.

Explant	Mode	Basal Media	Plant Growth Regulators	Other addenda	Frequency	Reference
			Fagopyrum tataricum			
Hypocotyl	Somatic embryogenesis	MS Medium	2-iP (10 mg/L), 2,4-D (1 -8 mg/L) , BA (0.1 -2 mg/L), KIN (0.2 mg/L, 1mg/L), TDZ (0.5mg/L, 2.0mg/L), IAA (0.1 -1 mg/L)	--	20 % - 90 %	Han *et al.,* 2011
Cotyledon (Co) & Hypocotyl (Hy);	Somatic embryogenesis	MS medium	2,4-D (2 mg/L), Kin (1 mg/L), BA (2 mg/L), IBA (1 mg/L)	--	55.77 %	Wang *et al.,* 2016
Hypocotyl Protoplast	Organogenesis	MS medium	NAA (2 mg/L) , BA(1 mg/L)	--	25%	Lachmann and Adachi, 1990
Hypocotyl	Organogenesis	MS medium	2,4-D (1 -4 mg/L), NAA (0.5-1.5 mg/L), BA (0.2 -1.5 mg/L)	--	73%	Hou *et al.,* 2015
			Fagopyrum dibotrys			
Nodal segment	Organogenesis	MS medium	2,4-D (1-2 mg/L), BAP(1 -6 mg/L), NAA (0.2 -1 mg/L), TDZ (0.2 mg/L, 0.5 mg/L), IBA(0.1 -1 mg/L), IAA (0.1 -1 mg/L)	--	90%	Chen *et al.,* 2012
Nodal segment	Organogenesis	MS medium	BA(0.5 -4 mg/L), IBA (0.5 -4 mg/L), IAA (0.5 -4 mg/L)	--	3.8 shoots /explant	Majid *et al.,* 2015
			Fagopyrum cymosum			
Immature Inflorescence	Organogenesis	B_5 Medium	NAA(0.2 -5mg/L), BA (1mg/L, 2 mg/L), 2,4-D (1-5 mg/L), IBA (1mg/L)	--		Takahata in 1988

Approaches in *In-vitro* regeneration of *Fagopyrum* via Somatic embryogenesis from Immature Zygotic Embryo:

Neskovic*et al.,* 1987 found that 6% sucrose is important for embryogenesis in *Fagopyrum esculentum*. Somatic embryos and buds from immature embryos were obtained maximally when immature zygotic embryos pretreated for 5 days with B5 salt solution, 2, 4-D (5mg/L) + Kin. (0.1mg/L) were incubated further on B5 medium supplemented with BAP (2.2mg/L) + IAA (0.17mg/L) They found that pretreatment with 2, 4-D increased the number of explants which gave rise to embryogenic tissue, but the continued presence of 2, 4-D was not essential for a morphogenic response.

From Cotyledon:

Woo *et al.,* 2000 reported somatic embryogenesis and plant regeneration from cotyledon tissues of *Fagopyrum esculentum*. Calli were induced with high frequency on MS medium supplemented with 2mg/L 2, 4-D and 0.2 mg/L Kin. Embryogenesis with 32% frequency was achieved on MS medium in the presence of BAP (2.0mg/L) and Kin. (0.2mg/L) with 3% sucrose. The somatic embryos were progressed and developed further on a maturation media, simply containing ½ strength MS basal salts, vitamins and 3% sucrose.

From Hypocotyls:

An indirect somatic embryogenesis via the development of proembryogenic cell complexes (PECC) was observed in the *in vitro* cultured hypocotyl explants of 4-5-day-old buckwheat *(Fagopyrum esculentum* Moench.) seedlings by Gumerova *et al.,* 2001, 2003. PECC development in this report on hypocotyls explants was shown to depend on culturing conditions, including 2, 4-D concentration and sucrose concentration, the period of explants exposure to 2, 4-D, and explants density. Hypocotyls of Tartary buckwheat were used as explants for regeneration via somatic embryogenesis by Han *et al.,* 2011. Callus induction rates of 86.6% and 90% were achieved in 2, 4-D (4.0mg/L) and BA (1.0mg/L) under dark, and 2, 4-D (2.5mg/L) and BA (1.5mg/L) under light conditions respectively. The globular and torpedo stage embryos progressed further and developed into plantlets with IAA (1.0mg/L), Kin. (1.0mg/L), BA (2.0mg/L) and TDZ (0.5mg/L). Kwon *et al.,* 2013 cultured hypocoytl explants of *Fagopyrum esculentum*Moench on MS medium having 3% sucrose with different combinations of auxins and cytokinins. A combination of BA (1.0mg/L) and 2, 4-D (2.0mg/L) gave the maximal callus growth. Embryogenic calli were induced on MS media with 1mg/L BA and variable concentrations of 2, 4-D. interestingly, they showed that 2, 4 D didn't have any significant effect on induction of embryogenic callus. From the embryogenic calli obtained, 5- 20% callus showed regeneration on MS medium containing IAA (2.0mg/L), Kinetin (1.0mg/L), BA (1.0mg/L). Plantlet conversion was obtained with higher frequency when embryogenic calli with embryos and organized shoot primordial were transferred to MS basal medium with 3% sucrose.

Recently, Wang *et al.,* 2016 reported somatic embryogenesis in two tartary buckwheat cultivars. The Yuanzi cultivar was better than Xichang in terms of morphogenic response and hypocotyls explants were found better than cotyledon explants for somatic embryogenesis based regeneration. Calli could be induced maximally (98.96%) in a MS based medium supplemented with 2 mg/L 2, 4-D and 1 mg/L Kin. MS medium fortified with 2mg/L BA and 1mg/L Kin. Induced plant regeneration in 55.77% calli. Roots were induced in regenerated shoots on a half strength MS medium supplied with 1mg/L IBA. 75% of the regenerated plantlets survived after transfer to soil. In this work, they also studied the morphogenic potential of cultures from these two cultivars for multiple shoot induction. In cv Yuanzi, 69.05 % of regenerating propagules could be induced for multiple shoots on MS medium with 3 mg/L BAP and 1 mg/L TDZ.

From Leaf and Stem:

Park *et al.,* 1999 reported 1.6 to 5.6% rate of plant regeneration from callus derived from leaf and no regeneration from stem derived calli. Regeneration was obtained in hormone free medium.

Regeneration via Organogenesis
From Cotyledon and Hypocotyl:
Yamane in early 70's studied *in-vitro* differentiation in Shinano1 cultivar of buckwheat. Seeds were subjected to callus induction on modified White basal medium supplemented with varying concentrations (1-10 mg/L) of 2, 4-D, either alone or in combination with Kin. (0.2, 1 mg/L). Similarly, varying concentrations of IAA (1, 5 mg/L) either alone or in combination with Kinetin (0.2, 1 mg/L) were used. Calli developed from hypocotyls and cotyledon tissues from these seedlings optimally either in 10mg/L 2, 4-D or in a combination of 10 mg/L 2, 4-D and 0.2 or 1 mg/L Kinetin. Proliferated calli were then transferred to fresh RM-1964 medium modified with addition of 15% coconut milk and 3g/l yeast extract for development of shoots and roots. Srejovic and Neskovic, 1981 reported that regeneration of plants via organogenetic mode can be achieved in calli derived from cotyledon tissues. In their attempt to induce organogenesis, shoots were obtained with a modified B5 medium containing combination of 2, 4-D (1mg/L) and Kin. (1mg/L), and rooting occurred when all hormones were omitted and B5 basal medium containing vitamins was used.

In-vitro regeneration protocol using hypocotyls of 5-day old seedlings of *Fagopyrum esculentum* was established by Jin*et al.,* 2002. This study showed that calli could be induced on MS medium containing 1.0 mg/L to 2.0 mg/L 2, 4-D and 1.5 mg/L BAP and a high frequency (over 80%) of shoot differentiation was obtained on MS medium supplemented with BAP (2mg/L) and Kin. (1mg/L). A combination of NAA (0.2mg/L) and IBA (0.2 mg/L) promoted the formation of roots. They reported 2, 4-D and BAP as the main factors that influence callus induction. Hypocotyl explants of *F. esculentum* and *F.tataricum* were cultured by Hou*et al.,* 2015 on MS medium with varying concentrations of 2, 4-D (1-4 mg/L) either alone or in combination with various levels of BA (0.5-1.5 mg/L) . A reasonably good frequency of regeneration with BA (1 mg/L) + NAA (1 mg/L) was observed in *F. esculentum* and with BA (0.5mg/L) and NAA (1mg/L) in *F. tataricum*.

Micropopagation of three buckwheat cultivars via indirect regeneration from cotyledon and hypocotyl, direct regeneration from nodal segments as well as cultivation of shoot apices was studied by Klcova and Gubisova in 2008. Whereas in case of indirect organogenesis with cotyledon and hypocotyls explants they reported no significant difference in regeneration frequencies over the media combinations tested, a sharp 2-3-fold increase in regeneration ability was noticed in the tested cultivars when nodal segments were subjected to 0.5-1.0 mg/L BA supplemented MS medium. In case of shoot apices also, the study showed no significant difference among basal media combinations tested. The study, however, showed that rooting was improved up to 95.6% by adding 1mg/L of IBA. Luthar and Marchetti, 1994 reported regeneration from mature cotyledon in *Fagopyrum esculentum*. Cotyledon and hypocotyl explants were used by Berbec and Doroszewska, 1999 to check the effect of TDZ on regeneration in three different cultivars of buckwheat. Among three, cultivar Kora showed best regeneration response when cultured on LS medium containing TDZ (0.1mg/L) in combination with IAA (0.05mg/L). In the work of Lee, 2009 with *F. esculentum*cotyledons, a concentration of 4 mg/L BAP showed optimal development of various stages of shoot organogenesis and additionally 7 mg/L of $AgNO_3$ improved shoot regeneration frequency up to 30%.

From Leaf and Petiole:
The induction of callus and subsequent differentiation and organogenesis were accomplished by Woo *et al.,* 2004 in *Fagopyrum esculentum* from leaf explants. Kin. and 2, 4-D were found to be ideal for callusing. Kin. (0.5mg/L) and BAP (2.0mg/L) produced embryogenic and organized nodular regions. Organogenic callus showed high regeneration of plantlets with BAP (2.0 mg/L) and Kin. (0.2 mg/L) and in contrast, embryoids developed into plants in hormone free ½ MS medium with 3% sucrose. However, with leaf petiole explants of *Fagopyrum esculentum*, most shoot regeneration was induced on MS medium containing Gamborg vitamins, BA, 2iP, 2, 3, 5-TIBA at 1mg/L each and sucrose 30g/L by Slawinska*et al.,* 2009.

From Node:
Three cultivars of *Fagopyrum esculentum* were examined by Kachonpadungkitti*et al.,* 2001 to induce *in-vitro* flowering. Nodal segments of 2 week- old seedlings were cultured in modified MS medium containing Kin. (0.021 mg/L) and 3% sucrose solidified with 0.8% agar. Various factors that affect flower induction were studied using nodal segments. Low concentration of Kinetin, short day (8 h photoperiod), agar (1%), Gellan gum (0.1%), medium with aeration membrane, NO^-_3 (30mM) as only nitrogen source, sucrose concentration (5% or 7%) improved flower induction. One of the cultivar produced 100% flower bud by 8th week. Direct regeneration was reported by Chen *et al.,* 2012. Adventitious buds were regenerated from stem nodal explants of *Fagopyrum dibotrys* in MS medium. 83% of explants developed axillary buds by BA (1.0mg/L) and 86% of shoots progressed further for development with BA (1mg/L) and NAA (0.5 mg/L). For rooting of the regenerated shoots, they noticed ½ MS medium containing NAA (0.5mg/L) was the best.

A regeneration protocol for *Fagopyrum dibotrys* through nodal explants was reported by Majid *et al.,* 2015. Increase in BAP concentration up to 3mg/L increased shoot propagation and further increase in concentration decreased the number of shoots. Optimal hormone for rooting was IBA (2.5mg/L) and IAA (2.0mg/L) in MS medium.

From Inflorescence:
Plant regeneration from immature inflorescence of common buckwheat was reported by Takahata in 1988. Direct shoot production from the inflorescence of common buckwheat was promoted by the addition of NAA (0.2mg/L) and BA (1mg/L) to B5 medium. MS medium with IBA (1.0mg/L) was employed for rooting.

From Anther:
Haploid plant regeneration through cultured anthers of common buckwheat has been reported by Bohanec*et al.,* 1993. They suggested that darkness preferably favors the regeneration on gellan-gum solidified media with 90g/L maltose, containing BA (2.5mg/L) and IAA (0.5mg/L). Anther culture was employed for plant regeneration of three Japanese cultivars by Yui and Yoshida, 2001. In two of the cultivars, the highest callus induction rate was attained on B5 basal medium with 2% sucrose supplemented with 1.0 mg/L NAA and 2.0 mg/L BA,

while a lower concentration of sucrose (1 %) and plant hormones (0.5 mg/I NAA and 1.0 mg/L BA) was good for the third cultivar. Cold pre-treatment of anthers promoted callus induction and some of the anther derived calli regenerated plantlets under callus inducing conditions in two of the cultivars. The regenerated plantlets were found to be diploid.

From Protoplast:

Adachi *et al.,* 1989 reported regeneration of *Fagopyrum esculentum* plants from protoplasts isolated from hypocotyls in MS medium supplemented with gibberellic acid (0.1mg/L). Continuous light with NAA (0.1mg/L) and BAP (0.2mg/L) gave the best response in shoot formation. Lachmann and Adachi, 1990 reported callus regeneration from hypocotyl protoplasts of *Fagopyrum tataricum* Gaertn. on MS medium containing 6% sucrose, BA (1.0mg/L) and NAA (1.0mg/L).

Genetic Transformation in Buckwheat

Although none of the studies mentions that *Fagopyrum* is recalcitrant to transformation (like legumes) or to *in-vitro* regeneration (like cotton), very few attempts have been made to develop transgenic plants of buckwheat. Only successful attempt to transfer a useful gene was the transformation of buckwheat with *AtNHX1,* a vacuolar Na(+)/H(+) antiporter gene from *Arabidopsis thaliana,* which was said to yield higher rutin content in transgenic buckwheat plants, thus offering a possibility to tolerate salt stress in a better way. Rest of the reports are related to method development. Some have developed *in-planta* transformation methods to circumvent the need of *in-vitro* culture or aseptic techniques and relatively faster analysis of transformation efficiency and expression of transgene in plant cell milieu. No study reports genetic transformation in buckwheat by particle bombardment method or via any of the direct-DNA delivery methods. A summarized view of some genetic transformation parameters in *Fagopyrum* sp. is presented in Table 2. Three types of approaches for genetic transformation in buckwheat are evident from the available studies:

Table 2: Explants, bacterial strains and vectors, genes, analyzing techniques and frequency

Transforming Agent	Explant	Strain : Vector	Gene	Detection Method	Frequency	References
A. tumefaceins **Involving tissue culture**						
	Cotyledon	A281 : pGA472	*nptII*	DNA hybridization and NPTII enzyme bioassays	Not mentioned , T1 segregated in 3:1 ratio	Djukic et al,1992
	Cotyledon, hypocotyl	LBA4404 : pHZX1	*AtNHX1*	PCR, Southern blotting, Northern blotting and RT- PCR	Hypocotyls : 24% Cotyledons: 6%	Chen *et al.,* 2007, 2008
	Hypocotyl	LBA4404 : pBI121	*gusA, nptII*	PCR, GUS assay	22-40 %	Kim *et al.,* 2001
In planta **transformation**						
	Apical meristem	LBA4404 : pBI121	*gusA, nptII*	PCR, Southern blotting	70%	Kojima *et al.,* 2000
	Plant	EHA105 : pCAMBIA 2301, pCAMBIA-PL	*gusA, nptII*	GUS by Fluorescence assay	Vacuum infiltration method has 57.3 fold higher efficiency than syringe infiltration	Bratic *et al.,* 2007
	Seed	GV3101 : pCAMBIA 1301	*gusA, hptII* gene	PCR	22.72 %	Chawla *et al.,* 2012
Agrobacterium rhizogenes						
Involving tissue culture	Stem	15834 : pBI121	*gusA, npt II*	PCR analysis, histochemical GUS assay and Northern hybridization	90%	Kim *et al.,* 2010
	Seeding explant	R1000, R1200, R13333, R15834, R1601, LBA9402, A4	*rol*A, B, C and D	PCR, HPLC	65-100%	Thwe *et al.,* 2016

In-vitro **Transformation and Regeneration:**

The susceptibility of common buckwheat to *Agrobacterium* species was checked by Neskovic et al as early as 1990. They found that tumors and hairy roots could be induced in *F. esculentum* Moench. by *A. tumefaciens* and *A. rhizogenes*, respectively. Out of several wild type strains of *A. tumefaciens* tested, strain A281 was found to be most virulent. Two years later, Djukic *et al.,* 1992 (from the same group) became the first to report a successful genetic transformation in buckwheat (*Fagopyrum esculentum* Moench.). Their procedure involved co-cultivation of cotyledon explants for 2 days with A281 strain of *Agrobacterium tumefaceins*, harboring *nptII*gene on pGA472 vector. DNA hybridization and NPTII enzyme bioassays were performed to validate transformants with resistance gene. Seeds from transgenic plants showed expected 3:1 segregation ratio when tested for kanamycin resistance in T_1 generation. Kim *et al.,* 2001 transformed hypocotyl explants of *F. esculentum* Moench. with *A. tumefaciens* LBA4404 harboring binary vector pBI121. A transformation frequency of 22-40% was achieved and transgenic plants were regenerated with a 4-8% frequency. Putative transformants were analyzed by GUS staining and fluorometric assay and regenerated plants were confirmed by dectection-PCR for *nptII* gene.

Agrobacterium-mediated method was used for transferring *AtNHX1*- tonoplast Na+ /H+ antiporter gene from *Arabidopsis thaliana* by Chen et al.2007, 2008 to regenerate salt-tolerant transgenic plants of *Fagopyrum esculentum*.

They utilized cotyledon and hypocotyl explants as well as LBA4404 strain of *Agrobacterium tumefacins* to study the effects of expressing *AtNHX1*genein common buckwheat. Study reported many factors affecting transformation like – preculturing of explants, time period of bacterial infection and co-cultivation. A higher transformation frequency was achieved in hypocotyls when pre-cultured for 1-2 days. The infection of cotyledon explants were carried out for 20 min. with bacterial culture and hypocotyl segments were infected for 25 min. A co-cultivation period of 1-2 days was found appropriate with both the type of explants for getting higher transformation frequency. Role of acetosyringone was not found to be very important in this study. Transformants were screened and validated by techniques like PCR, Southern blotting, Northern blotting and RT-PCR. The transgenic lines expressing *AtNHX1* gene were less affected by salt stress and had comparatively higher rutin content than the wild type and were shown to survive the salt stress regime as compared to control, non-transformed ones.

***In-planta* Transformation:**

Kojima *et al.,* 2000 exploited shoot apices of buckwheat to develop a meristem based *in-planta* transformation method by employing *Agrobacterium tumefaceins* (LBA4404, carrying pBI121). The procedure did not involve any *in-vitro* culture and newly established seedlings in soil pots with a pair of cotyledons were pricked with a needle to inoculate bacterial culture. The treated seedlings were co-cultivated at 22°C for 3 days and later grown under 16 hours photoperiod in a growth chamber. Plants were raised to maturity, flowered and selfed. T1 seeds were germinated in presence of geneticin and transformation frequency of 70% was calculated by detection PCR as analytical test. When checked for expression, 36% of the T1 seedlings showed resistance to geneticin. Further, mutants from T_1 having abnormal insertions were confirmed by southern blotting.

In planta transformation of buckwheat was later reported by Bratic *et al.,* 2007 by two different methods: vacuum infiltration and infiltration by syringe. *Agrobacterium tumefaciens* strain EHA105 with two vectors pCAMBIA2301 and pCAMBIA-PL was used. Outcomes of both methods were compared and it was stated that between the two methods vacuum infiltration is a preferable option for transformation in buckwheat as GUS activity was 57.3 folds higher than the value obtained in infiltration by syringe. *A.tumefaciens* culture density, vacuum conditions, and leaf maturity parameters were tested to make an effort on building up an easy transgenic plant development method in buckwheat. Chawla *et al.,* 2012 studied *in planta* transformation in tartary buckwheat with *Agrobacterium tumefaciens* (strain GV3101 harboring pCAMBIA 1301). Imbibed seeds were co-cultivated for 60 min. Integration of T-DNA was confirmed by histochemical GUS assay in leaf tissues of seedlings and PCR amplification of *hptII* gene. On an average, 22.72% transformation efficiency was noticed.

Transformation of Buckwheat for Enhanced Secondary Metabolite Production

Kim *et al.,* 2010 experimented with stem explants of buckwheat for inducing hairy roots and thereby obtained higher rutin production in transformed hairy root clones. *Agrobacterium rhizogenes* strain 15834 with vector pBI121 was co-cultivated with explants for 2 days to transfer neomycin phosphotransferase (*nptII*) and *gusA* genes. Results were confirmed by PCR analysis, staining for GUS activity and Northern hybridization. Rutin content was estimated by HPLC and it was shown that the amount of rutin content in hairy root clone was approximately 3-fold higher than the normal, wild type roots.

Thwe *et al.,* 2016 analyzed the transformation efficiencies of several *Agrobacterium rhizogenes* strains for induction of hairy roots in *F. tataricum* and investigated the expression levels of polypropanoid biosynthetic pathway genes in transformed hairy roots and also quantified the corresponding *in-vitro* synthesis of phenolic compounds and anthocyanins. For hairy roots in tartary buckwheat in this study, *A. rhizogenes* strain R1000 was found to be most efficient, as it showed highest transformation efficiency, highest growth rate, root number, root length and most importantly, highest anthocyanin and rutin content.

The effect of exogenous elicitors to enhance the flavonoids production was studied by Zhao *et al.,* 2014 in hairy root cultures of tartary buckwheat. Yeast polysaccharide elicitor stimulated the functional metabolite production in hairy roots and a combination of elicitation with yeast polysaccharide and medium renewal process was found to yield 3.2-fold higher flavonoids production. Huang *et al.,* 2016 was able to enhance the rutin and quercetin biosynthesis pathway genes in tartary buckwheat hairy root cultures by eliciting the hairy roots with UV-B radiation. They noticed a striking increase in rutin and quercetin production in UV-B stressed hairy roots.

Conclusion and Future Prospects

All the previous outcomes from plant tissue culture of *Fagopyrum* species confirm that there were potential in reestablishing new plant body from various explants of its three species viz. *F. esculentum, F. tataricum* and *F. dibotrys*. Although significant number of studies report *in-vitro* regeneration of buckwheat with varying degree of success and utilizing germplasms from many different temperate geographical regions, the fact remains that a high-frequency, reproducible protocol is the need of the hour for its genetic improvement, supporting its propagation at relatively greater scale and for widening its adoption as a food crop. Reports published from different countries and utilization of specific germplasms or cultivars show that significant degree of genotype dependency exists for *in-vitro* regeneration in buckwheat. One possible approach to solve this may be to identify a germplasm having better regeneration abilities and to work out a high frequency regeneration method and that germplasm should be made available to all the labs working in the area, so that more and more researchers put their augmented effort in genetic transformation with useful genes for improvement of this less adapted and under-exploited crop and later introgress the traits in their local germplasms. Similarly, for genetic transformation in buckwheat a protocol standardizing all the factors that affect genetic transformation viz. *Agrobacterium tumefaciens* strain, culture density at which culture is to be harvested for transformation, vector choices, type of explants, co-cultivation time period and temperature, requirement of Vir-inducing phenolic compounds is altogether missing. Also, strong and concerted efforts needed to improve this crop for tolerating several biotic and abiotic stress factors

and thus more studies are required on the deployment of stress tolerance genes in buckwheat. Enhanced production of secondary metabolites or other nutraceuticals utilizing hairy root culture and cell culture in buckwheat is also going to be an area where researchers can pay more attention. The present review can be utilized by researchers, institutions and agribiotech companies to sustainably increase the propagation of this useful plant commercially. Genetic engineering led crop improvement of buckwheat will soon also attract agriculture institutions, food biotech companies as well as pharma concerns to tap better resource yields from this plant.

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References

1. Adachi T, A Yamaguchi, Y Miike and E Hoffmann, Plant regeneration from protoplasts of common buckwheat (*Fagopyrum esculentum*), *Plant Cell Reports*, 8 (1989): 247-250.

2. Arora RK and JMM Engels, Buckwheat genetic resources in the himalayan region: Present status and future thrust, In Buckwheat Genetic Resources in East Asia, *International Crop Network,* 6 *(*1992): 87-91.

3. Berbec A and T Doroszewska, Regeneration *in vitro* of three cultivars of buckwheat *(Fagopyrum esculentum* Moench.) as affected by medium composition, *Fagopyrum,* 16 (1999): 49-52.

4. Betekhtin A, M Rojek, J Jaskowiak, A Milewska-Hendel, J Kwasniewska, Y Kostyukova, E Kurczynska, N Rumyantseva and R Hasterok, Nuclear genome stability in long-term cultivated callus lines of *Fagopyrum tataricum* (L.) Gaertn. *PLOS ONE*, 12.3 (2017): e0173537.

5. Bohanec B, M Neskovic and R Vujicic, Anther culture and androgenetic plant regeneration in buckwheat (*Fagopyrum esculentum* Moench), *Plant Cell Tissue and Organ Culture*, 35 (1993): 259-266.

6. Bratic A, DB Maji, JD Miljusjuki, ZS Jovanovi and V Maksimovi, *In planta* transformation of buckwheat (*Fagopyrum esculentum* Moench), *Archives of Biological Sciences*, 59 (2007): 135-138.

7. Campbell CG, Promoting the conservation and use of underutilized and neglected crops Buckwheat *Fagopyrum esculentum* Moench, 19 (1997): 27.

8. Chauhan RS, N Gupta, SK Sharma, JC Rana, TR Sharma and S Jana, Genetic and Genome Resources in Buckwheat - Present Status and Future Perspectives, *European Journal of Plant Science and Biotechnology*, 4.1 (2010): 33-44.

9. Chawla A, H Singh and A Kant , Development of In planta transformation method for Tartary Buckwheat (*Fagopyrum tataricum*), *International Conference on Emerging Trends in Biotechnology*, (2012): 45-46.

10. Chen LH, B Zhang and ZQ Xu, Genetic transformation of buckwheat (*Fagopyrum esculentum* Moench) with AtNHX1 gene and regeneration of salt-tolerant transgenic plants, *Chinese Journal of Biotechnology*, 23 (2007): 51-60.

11. Chen LH, B Zhang and ZQ Xu, Salt tolerance conferred by overexpression of *Arabidopsis* vacuolar Na (+)/H (+) antiporter gene AtNHX1in common buckwheat (*Fagopyrum esculentum*), *Transgenic Research*, 17 (2008): 121-32.

12. Chen C, J Lan, S Xie ,S Cui and A Li , *In vitro* propagation and quality evaluation of long-term micro-propagated and conventionally grown *Fagopyrum dibotrys* Hara mutant, an important medicinal plant, *Journal of Medicinal Plants Research*, 6.15 (2012): 3003-3012.

13. Djukic J M, M Neskovic, S Ninkovic and R Crkvenjakov, *Agrobacterium*-mediated transformation and plant regeneration of buckwheat (*Fagopyrum esculentum* Moench.), *Plant Cell Tissue and Organ Culture*, 29 (1992): 101-108.

14. Dutta MNM, S Shah and P Kumar, Assessment of agro-morphological, physiological and genetic diversity among buckwheat cultivars, *Proceeding of the 11th International Symposium on Buckwheat*, (2010): 94-101.

15. Fabjan N, J Rode, IJ Kosir, Z Wang, Z Zhang and I Kreft, Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) as a source of dietary rutin and quercitin, *Journalof Agriculture and Food Chemistry*, 51 (2003): 6452-6455.

16. FAO stats, 2017, http://www.fao.org/faostat/en/#compare

17. Gamborg OL, RA Miller and K Ojima, Nutrient requirements of suspension cultures of soybean root cells, *Experimental Cell Research*, 50 (1968): 151–158.

18. Greenway H and R Munns, Mechanisms of salt tolerance in nonhalophytes, *Annual Review of Plant Physiology*, 31 (1980): 149–190.

19. Gumerova EA, EI Gatina, SA Chuenkova and NI Rumyantseva, Somatic embryogenesis in common buckwheat *Fagopyrum esculentum* Moench, *Proceeding of 8th International Symposium on Buckwheat*, (2001): 377-381.

20. Gumerova EA, EI Galeeva, SA Chuyenkova and NI Rumyantseva, Somatic embryogenesis and bud formation on cultured *Fagopyrum esculentum* hypocotyls, *Russian Journal of Plant Physiology*, 50.5 (2003): 640–645.

21. Han MH, AHM Kamal, YS Huh, AY Jeon, JS Bae, KY Chung, MS Lee, SU Park, HS Jeong and SH Woo, Regeneration of plantlet via somatic embryogenesis from hypocotyls of *Tartary* Buckwheat (*Fagopyrum tataricum*), *Australian Journal of Crop Science*, 5.7 (2011): 865-869.

22. Hou S, Z Sun, B Linghu, Y Wang, K Huang, D Xu and Y Han, Regeneration of buckwheat plantlets from hypocotyl and the influence of exogenous hormones on rutin content and rutin biosynthetic gene expression in vitro, *Plant Cell Tissue and Organ Culture*, 120.3 (2014): 1159-1167.

23. Huang X, J Yao, Y Zhao, D Xie, X Jiang and Z Xu, Efficient Rutin and Quercetin biosynthesis through Flavonoids-related gene expression in *Fagopyrum tataricum* Gaertn. Hairy Root Cultures with UV-B Irradiation, *Frontiers in Plant Science*, 7 (2016): 63.

24. Jin H, J Jia and J Hao, Efficient plant regeneration *in vitro* in buckwheat, *Plant Cell Tissue and Organ Culture* 69 (2002): 293–295.

25. Joshi BD and RS Rana, Buckwheat (*Fagopyrum esculentum*), In Underutilized crops: Cereals and Pseudocereals, Ed. Williams, J.T. Chapman and Hall, London, (1995): 85-127.

26. Kachonpadungkitti Y, S Romchatngoen, K Hasegawa and S Hisajima, Efficient flower induction from cultured buckwheat (*Fagopyrum esculentum*L.) node segments *in vitro*, *Plant Growth Regulation*, 35 (2001): 37–45.

27. Kim YK, H Xu, WT Park, NI Park, SY Lee and SU Park, Genetic transformation of buckwheat (*Fagopyrum esculentum* M.) with *Agrobacterium rhizogenes* and production of rutin in transformed root cultures, *Australian Journal of Crop Science*, 4.7 (2010): 485-490.

28. Klcova L and M Gubisova, Evaluation of different approaches to Buckwheat (*Fagopyrum esculentum*Moench.) micropropagation, *Czech Journal of Genetics and Plant Breeding*, 44.2 (2008): 66–72.

29. Kojima M, Y Arai, N Iwase, K Shirotori, H Shioiri and M Nozue, Development of a simple and efficient method for transformation of buckwheat plants (*Fagopyrum esculentum*) using *Agrobacterium tumefaciens*, *Bioscience, Biotechnology and Biochemistry*, 64 (2000): 845-847.

30. Kreft I, N Fabjan and K Yasumoto, Rutin content in buckwheat (*Fagopyrum esculentum* Moench) food materials and products, *Food Chemistry*, 98 (2006): 508–512.

31. Kwon SJ, MH Han, YS Huh, SK Roy, CW Lee and SH Woo, Plantlet regeneration via somatic embryogenesis from hypocotyls of common buckwheat (*Fagopyrum esculentum* Moench.), *Korean Journal of Crop Science*, 58.4 (2013): 331-335.

32. Lachmann S and T Adachi, Callus regeneration from hypocotyl protoplasts of *tartary* buckwheat *(Fagopyrum tatericum* Gaertn.) *Fagopyrum,* 10 (1990): 62-64.

33. Lee SY, YK Kim , MD R Uddin, NI Park and SU Park , An efficient protocol for shoot organogenesis and plant regeneration of buckwheat (*Fagopyrum esculentum*), *Romanian Biotechnological Letters*, 14.4 (2009) : 4524-4529.

34. Linsmaier EM and F Skoog , Organic growth factor requirements of tobacco tissue culture, *Plant Physiology*, 21 (1965): 487-492.

35. Luthar Z and S Marchetti, Plant regeneration from mature cotyledons in buckwheat *(Fagopyrum esculentum* Moench) germplasm collection, *Fagopyrum,* 14 (1994): 65 – 69.

36. Majid A, ZA Kaloo , BM Padder and AH Munshi, An efficient *in vitro* regeneration protocol for an endandered medicinally important herb *Fagopyrum dibotrys* growing in Kashmir Himalaya, *CIBTech Journal of Biotechnology*, 4.1 (2015): 2319–3859.

37. Murashige T and F Skoog, A revised medium for rapid growth and bioassay of tobacco tissue culture. *Physiologia Plantarum*, 15 (1962): 473-497.

38. Neskovic M, R Vujicic and S Budimir, Somatic embryogenesis and bud formation from immature embryos of buckwheat (*Fagopyrum esculentum* Moench.), *Plant Cell Reports*, 6 (1987): 423-426.

39. Neskovic M, B Vinterhalter, J MiljusDjukic, S Ninkovic, D Vinterhalter, VJovanovic and J Knezevic, Susceptibility of buckwheat (*Fagopyrum esculentum* Moench.) to *Agrobacterium tumefaciens* and *A. rhizogenes, Fagopyrum,* 10 (1990): 57-61.

40. Ohnishi, Discovery of new *Fagopyrum* species and its implication for the studies of evolution of *Fagopyrum* and of the origin of cultivated buckwheat, *Proceeding of the 6th International Symposium on Buckwheat*, (1995): 175-181.

41. Park CH, SL Lee , CS Chung, YB Shin, NS Kim, YB Kim, KM Yoon and YS Choi, Somatic embryogenesis and plant regeneration from leaf and stem explants of buckwheat, *Fagopyrum,* 16 (1999):53-56.

42. Park CH, YB Kim, YS Choi, K Heo, SL Kim, KC Lee, KJ Chang and HB Lee, Rutin content in food products processed from groats, leaves, and flowers of buckwheat, *Fagopyrum,* 17 (2000): 63-66.

43. Rajbhandari BP, S Dhaubhadel, DM Gautam, and BR Gautam, Plant regeneration via calli of leaf and stem explants in common buckwheat ecotypes, *Current Advances in Buckwheat Research*, (1995): 191-196.

44. Slawinska J, SK Kantartzi and RL Obendrof , *In vitro* organogenesis of *Fagopyrum esculentum* Moench (*Ploygonaceae*) as a method to study seed set in Buckwheat, *European Journal of Plant Science and Biotechnology*, 3 (2009): 75-78.

45. Srejovic V and M Neskovic, Regeneration of plants from cotyledon fragments of buckwheat (*Fagopyrum esculentum*Moench). *Zeitschrift fur Pflanzenphysiologie*, 104 (1981): 37-42.

46. Suvorova G, Buckwheat tissue cultures and genetic transformation, *Molecular Breeding and Nutritional Aspects of Buckwheat*, (2016): 365.

47. Takahata Y, Plant regeneration from cultured immature inflorescence of common buckwheat (*Fagopyrum esculentum* Moench) and perennial buckwheat (*F. cymosum* Meisn.), *Japaneses Journal of Breeding*, 38 (1988) 409-413.

48. Thwe A, MV Arasu, X Li , CH Park, SJ Kim, NA Al-Dhabi and SU Park , Effect of different *Agrobacterium rhizogenes* strains on hairy root induction and phenylpropanoid biosynthesis in tartary buckwheat (*Fagopyrum tataricum* Gaertn), *Frontiers in Microbiology*, 7 (2016): 318

49. Wang CL, XN Dong , MQ Ding, YX Tang, XM Zhu, YM Wu, ML Zhou and JR Shao, Plantlet regeneration of tartary buckwheat (*Fagopyrum tataricum* Gaertn.) *in vitro* tissue cultures, *Protein and Peptide Letters*, 23.5 (2016): 468-477.

50. White PR, The Cultivation of Animal and Plant Cells, *Science*, 1/e, Ronald Press, New York, (1954): 239.

51. Woo SH, A Nair, T Adachi and CG Campbell, Plant regeneration from cotyledon tissues of common buckwheat (*Fagopyrum esculentum* Moench), *In Vitro Cellular and Developmental Biology- Plant*, 36 (2000): 358-361.

52. Woo SH, M Takaoka , HS Kim, CH Park, T Adachi and SK Jong , Plant regeneration via shoot organogenesis from leaf callus culture of common buckwheat (*Fagopyrum esculentum* Moench.), *Proceedings of the 9th Inernationall Symposium on Buckwheat*, (2004): 61-65.

53. Yamane Y, Induced differentiation of buckwheat plants from subcultured calluses *in vitro*, *Japanese Journal of Genetics*, 49.3 (1974): 139-146.

54. Yui M and T Yoshida, Callus induction and plant regeneration in anther culture of Japanese buckwheat cultivars (*Fagopyrum esculentum* Moench), *Fagopyrum, 18* (2001): 27-35.

55. Zhao JL, L Zou , CQ Zhang , YY Li, LX Peng, DB Xiang and G Zhao, Efficient production of flavonoids in *Fagopyrum tataricum* hairy root cultures with yeast polysaccharide elicitation and medium renewal process, *Pharmacognosy Magazine,* 10.39 (2014): 234.

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