RESEARCH NOTE

Hybridization Technique in Tartary Buckwheat (*Fagopyrum tataricum* Gaertn)

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Nepalese people called two cultivated species of Fagopyrum (*F. esculentum* and *F. tataricum*) collectively Phapar (Buckwheat). Common buckwheat (*F. esculentum*) is called Mithe Phapar and tartary buckwheat (*F. tataricum*), Tite Phapar in Nepali. Buckwheat is cultivated from 60 to 4200 m altitude and in 61 districts of Nepal (Baniya 1999, Vaidya et al 1999, Joshi 2008). Farmers value buckwheat as vegetable, food and medicinal crop (Baniya 1999, Vaidya et al 1999). Because of its unusual and very unique behavior as well as medicinal and economical values, its potential is very high for improving farmers' livelihoods. Buckwheat is a high value crop however, research effort has been only limited to introduction and simple phenotypic selection in Nepal. Lately hybridization followed by genotypic selection is initiated in Biotechnology Unit, Khumaltar to develop high yielding, bitterless and non-adhering hull type varieties.

Tartary buckwheat (*Fagopyrum tataricum* Gaertn, 2n = 2x = 16) is a staple food crop in Mountain regions (agroecologically, High Hill) of Nepal. It can be grown in summer season in High Hill, autumn and spring seasons in Mid Hill and winter season in Low land (Tarai). Cultivating areas of buckwheat have been increasing throughout the country because of its easiness of cultivation, high medicinal and environmental protection values. Breeding work is limited only to selection on existing natural variation mainly due to very difficult to produce F_1 seeds among the landraces of tartary buckwheat. Flower is inconspicuous, incomplete, bisexual and cleistogamy in nature. Therefore, it is extremely difficult to emasculate manually. Only Mukasa et al (2007) and Wang and Campbell (2007) produced F_1 seeds among tartary buckwheat. With the hot water aided emasculation, we produce F_1 seeds among Tartary buckwheat first time in Nepal.

Eighteen accessions of tartary buckwheat (Table 1) were taken from Gene Bank of NARC and grown in buckwheat in Greenhouse in 2008. Staggered planting, though not necessary because of indeterminant growth habit, was done for synchronized flowering. Three methods namely hand emasculation, hot water using Thermos and hot water using electric water bath (Thermo Minder SD Mini, TAITEK Corporation, Japan) were randomly used for emasculating these genotypes. Flower clusters were prepared by removing opened flower, ready to open flower and very immature and seed set flower. On an average there were 6 flowering buds in a cyme. Emasculation was done from 2-5 pm.

Mostly the shiny small flower buds which were not longer than bract length were taken to emasculate (Figure 2). For hand emasculation, top portion about $1/3^{rd}$ of flowering bud were cut and in some cases, hand emasculation was done without cutting the top portion. 8 anthers were removed by needle or forceps with help of magnifying lens. Different temperatures at different time period of soaking flower buds were tested in Thermos and Water bath and these treatments are given in Table 1. Thermometer was used to check the water temperature and stop watch to check the soaking time

duration. In the next day, opened flowers were marked and used for pollination. Two methods, rubbing and brushing were followed for pollination with the help of magnifier. Anthers were collected from the flowering buds ready to open in petridish and pollens were released pressing the anther by needle. These pollens were dusted to emasculated flower by brush or needle. In some cases, anther were picked by forceps and rubbed to the stigma. Open flowers from the male parent were taken by forceps and rubbed in the emasculated flowers in the morning with the help of magnifier. Pollinated flowers were regularly monitored and one week after treatment, number of seeds and dead flowers were counted.

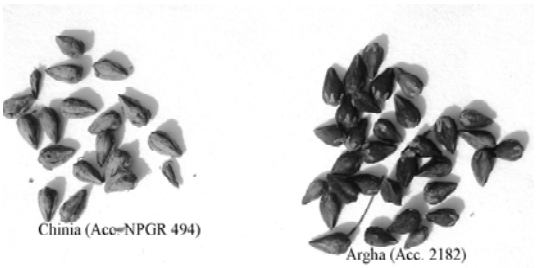
Table 1. Parental lines used in crossing (CN, crossing number) and treatment for emasculation

CN	Female parent		Male parent	Emasculation	
	Name	Accession		Method	Treatments (^{o}C-min)
1	Andheri	NPGR-5663	Kabre Tite	Hand	-
2	Argha	NPGR-2182	Bijuwar	Hand	-
3	Bijuwar	NPGR-2191	Kabre Tite	Thermos	45-3
4	Chalsa-1	NPGR-5658	Bijuwar	Thermos	44-3
5	Chinia	NPGR-494	Argha	Water bath	I. 45-3, II. 44-3
6	Chyangboche	NPGR-2237	Jumla Tite-1	Thermos	44-2
7	Dablyang	NPGR-5671	Sample#8	Thermos	43-5
8	Dolpa Tite	NPGR-11329	Kabre Tite	Thermos	42-5
9	Ghiling Tite	NPGR-11385	Argha	Water bath	I. 44-3, II. 44-2
10	Jumla Tite-1	NPGR-11358	Kabre Tite	Water bath	43-3
11	Jumla Tite-2	NPGR-11363	Kabre Tite	Water bath	I. 43-3, II. 42-4
12	Kabre Tite	NPGR-11322	Khalde-2	Thermos	45-3
13	Khalde-2	NPGR-5668	Argha	Thermos	44-3
14	Khinga	NPGR-8207	Khalde-2	Hand	-
15	Morudung	NPGR-2224	Khinga	Hand	-
16	Sample#8	NPGR-1319	Chalsa-1	Thermos	42-4
17	Sirdibas	NPGR-2214	Dolpa Tite	Hand	-
18	YDR-2-FT-19-4	NPGR-11304	Jumla Tite-1	Hand	-

Flowers emasculated by hand did not open and force pollinated (Table 2). All flowers were died. Every flower parts are very sensitive and delicate. Therefore after touching them by forceps or needles, they turned dried. After cutting the tips, flower turned dark and did not open completely. Hand emasculation is very difficult and could not successful in tartary buckwheat. Similarly flowers emerged in Thermos hot water did not open. Forced pollination was followed in such case. However, flowers could not set the seeds. It is noticed that in Thermos, temperature could not be maintained at right degree. Deeper the Thermos more the temperature. High temperature inhibit flower to open (Mukasa et al 2007).

Most of the flowers opened next day after treating in water bath. This is because; the temperature in the water bath is constant. Very few F_1 seeds have been produced using hot water in water bath for emasculation. This method is seemed very useful and simple for producing F_1 seeds. After crossing, different characters can be used for testing F_1 seeds. Cotyledon color in just after emergence, seed shape, maturity, hull color, etc will be useful but the inheritance patterns of these characters are necessary. In addition to this morpho type of F_1 seeds or plant, segregation pattern in F_2 supplement the successful of crossing. For example, dark red cotyledon color is controlled by single recessive gene. Non adhering hull is the output of single recessive gene and independent with hull color. Dark hull color is controlled by dominant gene (Mukasa et al 2007).

F_1 seeds were successfully produced first time in Nepal by crossing two landraces {Chinia (NPGR 494) / Argha (NPGR 2182)} using hot water method for emasculation. F_1 seeds were produced only from the flower treated at 44^{o}C for 3 minute.



Seeds of two parents used in crossing

Flower cluster after removing unnecessary flowers and seeds

Apical part soaking in hot water for emasculation

Flower opened in the following day of hot water treatment

Figure 2. Seeds of parents and emasculation process.

Table 2. Emasculation and pollination details along with seed set

CN	Flower/bud, n		Pollination		Seed set, n
	Emasculated	Opened	Method	Flower pollinated , n	
1	3	-	Rubbing	3	0
2	4	-	Brushing	4	0
3	35	0	Brushing	0	0
4	45	0	Brushing	0	0

5	I. 44, II. 56	I. 0, II. 11	Rubbing	I. 0, II. 6	I. 0, II. 2
6	60	0	Rubbing	0	0
7	56	0	Brushing	0	0
8	34	2	Brushing	2	0
9	I. 34, II. 16	I. 8, II. 3	Rubbing	I. 3, II. 1	I. 1, II. 0
10	60	14	Rubbing	5	0
11	I. 20, II. 25	I. 4, II. 5	Rubbing	I. 2, II. 2	I. 0, II. 0
12	24	0	Brushing	3	0
13	15	0	Rubbing	4	0
14	6	-	Rubbing	6	0
15	7	-	Rubbing	7	0
16	24	1	Rubbing	3	0
17	4	-	Brushing	4	0
18	2	-	Brushing	2	0

Refer Table 1 for crossing number.

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