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Short Communication

Efficiency and utility of pollination without emasculation (PWE) method in intra- and inter-specific hybridization in soybean

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Abstract

Efficiency and applicability of a new method of hybridization called 'pollination without emasculation' was evaluated in a set of crosses involving *Glycine max* and *Glycine soja* genotypes. The genotypes were crossed in various combinations to develop MAGIC (multiparent advanced generation intercross) and NAM (nested association mapping) population as well as to widen the genetic base of soybean through wide hybridization. Success of hybridization ranged from 33 % to 77% during *kharif* season. Number of false crossed seeds as measured through hybridity testing was negligible (0-15%). Rate of crossing success was more during *kharif* than *rabi* (17.33%) season. The PWE appeared to be highly efficient and applicable equally to intra-specific and inter-specific crosses in soybean.

Key words: hybridization, emasculation, pollination, soybean

Success of soybean breeding has been impeded to a large extent by the poor success rate of hybridization. Soybean flower is small, delicate and highly sensitive to injury to the stigma. In general, success rate of soybean hybridization ranges from 2-3% to 11-15% depending upon crossing approach followed and environmental condition prevailed where the crop is grown. Usually, two different approaches are followed for hybridization in soybean, *viz*., 1) emasculation in the afternoon followed by pollination in the next day morning; 2) emasculation followed by pollination in the morning (Agrawal et al. 2001). Both the approaches are tedious, time consuming and poorly efficient as most of the crossed flower buds drop due primarily to injury caused during emasculation. Talukdar and Shivakumar (2012) reported an improved method of soybean hybridization where pollination was done without emasculation; hence the method was called "pollination without emasculation" (PWE). In this method, a flower bud in right stage is selected; petals are opened slightly to locate the stigma and pollination is effected by dusting the stigma with pollens from the male flower. Using this technique, success of soybean hybridization has been raised from 2-3% to 35-39% in Delhi. The method was successfully utilized to transfer the null allele of Kunitz trypsin inhibitor (kti) from PI542044, an exotic line, to DS9712 (a popular Indian variety) through marker-assisted backcross breeding approach (Talukdar et al. 2014). In the present study, utility and efficiency of this technique was studied in a large scale hybridization program undertaken at the ICAR-Directorate of Soybean Research (DSR), Indore (Madhya Pradesh).

Indian soybean genotypes are considered to be poorly diverse. The soybean gene pool of India is primarily consisted of genotypes introduced from Taiwan and the United States of America. Soybean hybridization therefore, remain confined mainly to those few introduced lines. The Indian breeders appear to be biased towards a few selected genotypes which are being used repeatedly in the crossing programs (Tiwari, 2014). Uses of wild type soybean (*Glycine*

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soja, *Glycine tomentolla*) in hybridization remained to be negligible. As a result, the genetic base remained narrow and poor, arresting the progress of soybean breeding in India. To overcome this bottleneck and to widen the genetic base, large scale hybridization program involving both cultivated and wild type soybean has been started in different institutes including Indian Agricultural Research Institute, New Delhi. Further, to develop next generation mapping population *viz*., multi-parent advanced generation inter-cross (MAGIC) and nested association mapping (NAM), crossing program has been initiated in DSR, Indore. Such populations would be useful in mapping quantitative trait loci (QTL) for various traits of economic importance in soybean (Bandillo et al. 2013; Talukdar and Talukdar, 2013). In these crossing programs, PWE method of hybridization was used and its efficiency and applicability in intra-specific and inter-specific crosses was evaluated.

For development of MAGIC population, four popular Indian soybean varieties viz., JS335, JS9560, NRC37 and NRC86, and four exotic germplasm lines *viz*., EC572109, EC572136, EC546882 and EC333901 were used to develop hybrids. Success of hybridization varied among the cross combinations ranging from 54.23% to 66.25%. In total, 254 pods were harvested from 440 buds crossed representing 57.72% success of hybridization (Table 1). Similarly, for the development of NAM population, a popular variety JS9560 was used as reference line and was crossed pair-wise with six founder line viz., JS335, NRC37, NRC86, EC546882, EC333901 and EC572136. Success of hybridization in NAM crosses ranged from 27.27% to 56% with an average of 42.07%. In the crosses involving wild type genotype, the success rate of hybridization was 50%. Similar trend of success was observed in other cross combinations (Table 1). Considering the entire cross combinations together, the success of crossing was found to be 51.90%. This value was significantly higher than that has been reported earlier by Talukdar and Shivakumar, 2012. This higher success rate can be attributed to the weather conditions prevailing during crossing period in Indore where these crosses were performed. Compared to New Delhi, the weather in Indore remains mild, cool and humid during flowering period of soybean. Moreover, crossing could be performed for longer period of time due to prevailing favorable weather. In Indore, hybridization could be performed from 7:30am in the morning to 1:00 pm in the afternoon, while in Delhi crossing has to be over between 8:30 to 10:30 am. Due to mild weather with lower wind speed in Indore, flower drop was also observed to be low. Thus, the PWE technique found to perform better in a better environment.

Soybean is a highly self-pollinated crop. Effect of artificial hybridization is therefore not known until the plants generated from the crossed seeds are tested for hybridity. Phenotypic traits *viz*., hypocotyls coloration, flower color, pubescent type/coloration, etc. can be used to seperate a hybrid from a self-fertilized plant. Simple sequence repeat (SSR) markers can also

Table 1. Success of PWE method of hybridization during *kharif* 2014

Cross combination	No. of buds pollinated	No. of buds produced matured pods#	No. of seeds germi-nated	No. of hybrid plants$
MAGIC population				
EC72109 x JS9560	130	71(54.61)	78	74(94.87)
EC72136 x JS335	118	64(54.23)	35	35(100)
EC546882 x NRC37	80	53(66.25)	71	69(97.18)
EC333901 x NRC86	112	66(58.92)	78	77(98.72)
NAM population				
EC333901 x JS9560	51	20(39.21)	14	12(85.71)
NRC86 x JS9560	25	14(56.00)	12	11(91.67)
EC572136 x JS9560	22	6(27.27)	1	1(100)
EC46882 x JS9560	30	14(46.66)	13	12(92.31)
NRC37 x JS9560	24	10(41.66)	12	12(100)
JS335 x JS9560	12	5(41.60)	1	1(100)
Wide hybridization				
JS9560 x *Glycine soja*	37	24(64.86)	23	23(100)
NRC86 x *G soja*	16	5(31.25)	4	4(100)
NRC37 x *G soja*	19	7(36.84)	4	4(100)
EC572109 x *G soja*	3	1(33.33)	2	2(100)
Rust resistance introgression				
JS335 x EC241780	7	4(57.14)	4	4(100)
JS9560 x EC241780	3	1(33.33)	0	0
NRC37 x EC241780	2	1(50.00)	3	3(100)
NRC86 x EC241780	3	2(66.66)	2	2(100)
Yield enhancement				
EC 572109 x JS335	33	8(24.24)	4	4(100)
EC333901 x EC572109	9	7(77.00)	2	2(100)
EC572109 x NRC86	9	6(66.66)	1	1(100)
EC586882 x EC333901	2	1(50.00)	1	1(100)
EC333901 x NRC86	3	1(33.33)	1	1(100)
EC546882 x NRC86	4	2(50.00)	3	3(100)
EC572109 x EC333901	9	3(33.33)	5	5(100)
Total	763	396(51.90)	374	363(97.06)

#Figure in parentheses indicate percent success of crosses.
$Figure in parentheses indicates percent true hybrids

be used effectively and efficiently to test hybridity of a putative hybrid plant (Fig. 1). In this experiment, it was found that the seeds harvested from the crossed flower buds produced mostly hybrid plants, which ranged from 95-100% (Table 1). This is the true indicator of potentiality of this technique of hybridization. Higher success of PWE can be attributed primarily to the selection of correct flower buds, collection of mature pollen, and inducing least injury to the floral parts, stigma in particular. In fact, soybean flowers are protogynous in nature i.e. the stigma becomes receptive at least 24 hours before the pollen of the same flower become matured enough to fertilize it. Therefore, emasculation prior to pollination can be avoided without any fear of self-fertilization provided a bud in right stage is selected.

Usually, *rabi* season is least preferred for effecting crosses in soybean due to dry weather condition that normally prevails during that period of the year. However, in order to affect four-way crosses and expedite development of MAGIC population, 115 F_1 seeds from cross combinations *viz*., EC572109 x JS9560, EC572136 x JS335, EC546882 x NRC37 and EC333901 x NRC86 were grown during *rabi* 2014. Of the 115 seeds, 89 germinated of which 87 were tested to be true hybrid. Flower of these hybrid plants were used to make 4-way crosses. Of the six cross combinations, two got damaged by pests and diseases. Hence, 15 pods from115 crossed-buds in 4 cross-combinations were harvested keeping success rate @ 13.04%. Considering weather condition, this rate of success is nothing unusual as success of hybridization during *rabi* season is always less than *kharif* season. Talukdar and Shivakumar (2012) also made similar observation of poor crossing success during *rabi* in New Delhi. Therefore, crossing is advised to effect either in *kharif* season, which is main season for soybean in India, or in controlled environmental condition. Good success of hybridization was recorded in National Phytotron Facility, New Delhi.

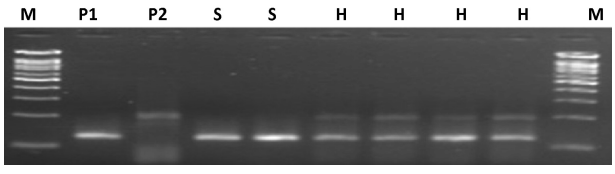

Fig. 1. SSR marker-assisted hybridity testing of putative soybean hybrid plants. Plants with two bands are heterozygous and true hybrids. M: Marker; P1: Female parent; P2: Male parent; S: Self-fertilized plants; H: Hyrbid plants

Table 2. Success of hybridization in 4-way intercross during *rabi* 2014

Cross combinations	No. of buds pollinated	No. of buds produced matured pods	Percent success of crosses
[EC546882 / NRC37] x [EC572136/JS335]	27	5	18.51
[EC546882/NRC37] x [333901/NRC86]	29	4	13.79
[EC546882/NRC37] x [EC572109/JS9560]	14	1	7.14
[EC333901/NRC86] x [EC572136/JS335]	45	5	11.11
Total	115	15	13.04

The result of the present study has convincingly established that PWE is suitable for hybridization in both cultivated and wild type soybean. Further, efficacy of this is far better when it is used in areas with favorable climatic conditions. Large scale application of this technique would help the breeders to generate various breeding materials involving both cultivated and wild type genotypes of soybean.

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