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Production and characterization of inter and intraspecific hybridization eggplant

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ABSTRACT

The eggplant is a highly valuable horticultural crop grown all over the world and it is of substantial economic importance in Asia. However, its production is severely threatened by several soil-borne and foliar diseases, insect-pests, drought, heat, and frost damage. Therefore, efforts to transfer useful resistance genes into eggplant from their wild relatives is important. In the present study, interspecific and intraspecific hybridization was carried out, that included three cultivated genotypes of eggplant (*Solanum melongena* MEE, *Solanum melongena* MEP, *Solanum melongena* MEB) and one wild *Solanum* species (*Solanum incanum* INC). The F₁ hybrids were made by inter and intraspecific hybridization. A total of 632 possible inter and intraspecific reciprocal crosses was performed where only three were successful. The minimum days to flowering were observed in parent MEP, and maximum plant height was measured in MEE×MEB. Maximum fruit length was observed in parent MEB. Furthermore, fruit diameter, leaf width, leaf length, and fruit yield per plant were found maximum in hybrid MEE×INC. Our results suggest that these materials will be of great interest for the genetic improvement of eggplant; they may have a tremendous potential to increase tolerance to abiotic stresses, such as to drought and heat, as well as increased nutrient and herbal values. Findings of this study will be helpful for the human health, ultimately contributing to the development of a new generation of plants adapted to climate.

Keywords: *Solanum melongena*, *Solanum incanum*, Solanaceae, hybrid, heterosis, yield, wild species.

RESUMO

Produção e caracterização de berinjela de hibridização inter e intraespecífica

A berinjela é uma cultura hortícola muito valorizada, cultivada em todo o globo e de grande importância econômica na Ásia. No entanto, sua produção está ameaçada por várias doenças foliares ou transmitidas pelo solo, pragas, seca, calor e danos causados por geada. Portanto, esforços são importantes para transferir genes de resistência para a berinjela, provenientes de seus parentes selvagens. No presente estudo, foi realizada hibridização interespecífica e intraespecífica, que incluiu três genótipos cultivados de berinjela (*Solanum melongena* MEE, *Solanum melongena* MEP, *Solanum melongena* MEB) e uma espécie selvagem de *Solanum* (*Solanum incanum* INC). Os híbridos F₁ foram produzidos por hibridização inter e intraespecífica. Um total de 632 cruzamentos recíprocos inter e intraespecíficos possíveis foram realizados, onde apenas três tiveram sucesso. Os dias mínimos para o florescimento foram observados na MEP parental, e a altura máxima da planta foi medida em MEE×MEB. O comprimento máximo do fruto foi observado na MEB parental. Além disso, o diâmetro do fruto, a largura da folha, o comprimento da folha e a produção de frutos por planta foram encontrados máximos no híbrido MEE×INC. Nossos resultados sugerem que esses materiais serão de grande interesse para o melhoramento genético da berinjela; eles podem ter enorme potencial para aumentar a tolerância aos estresses abióticos, como seca e calor, bem como aumentar os valores nutricionais. Os resultados encontrados neste estudo serão úteis para a saúde humana, em última análise, contribuindo para o desenvolvimento de uma nova geração de plantas adaptadas ao clima.

Palavras-chave: *Solanum melongena*, *Solanum incanum*, Solanaceae, híbrido, heterose, produção, espécie selvagem.

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Relative wild species can contribute to spread the genetic background of crops and adapt them to new challenges, such as climate change, tolerance to abiotic and biotic stresses (Dempewolf *et al.*, 2014). The utilization of wild relatives in crop breeding has economic

impact at the global level in 164.5·10⁹ US\$ annually. Current improvement of the crops by using wild relatives in breeding has gained more importance in the climate change scenario besides conferring resistance to different diseases (Tyack & Dempewolf, 2015).

This clearly shows how research in crop wild relatives and its utilization in breeding may have an important economic impact by developing new cultivars with improved characteristics.

One of the vegetables, in which significant efforts are being done in

the last years for interspecific breeding from related species for adaptation to climate change, is the common eggplant (*Solanum melongena*) (Liu *et al.*, 2015; Plazas *et al.*, 2016). The eggplant belongs to the Solanaceae family, which is a highly valuable horticultural crop grown all over the world and it is of substantial economic importance in Asia, Africa, and the subtropics (Collonnier *et al.*, 2001). It is a good source of proteins, minerals, and antioxidant and also has medicinal value (Magioli & Mansur, 2005). However, its production is severely threatened by several soil-borne and foliar diseases, insect-pests, drought, heat and frost damage, which reduce yield (Collonnier *et al.*, 2001). Therefore, efforts to transfer useful resistance genes into eggplant from the wild relatives are important because of the lack of resistance in common eggplant gene pool.

Eggplant wild species (*Solanum incanum*, also known as Sodom or thorn apple) is found growing naturally in both tropics and subtropics (Waweru *et al.*, 2017) and has been regarded as wild progenitor of *Solanum melongena* (Prohens *et al.*, 2013). It is herbaceous or a soft wooded shrub having spiny stem and leaves covered with velvety hairs. Its extract is used to control ticks in cattle (Mwaura *et al.*, 2011). Moreover, its fruit has been used to cure mycotic infections in Africa. Compost is also prepared by this wild plant (Mwaura *et al.*, 2011). Furthermore, extract of its fruit has been used to control nematode (*Meloidogyne* spp.) in *Capsicum annum* (Waweru *et al.*, 2017) and so it can be used as an environmentally safe nematicide. It is believed to contain high quantity of phenolic compounds and therefore can be used in improvement of eggplant quality through breeding (Prohens *et al.*, 2013). However, Kaushik *et al.* (2016) reported small fruit size of the hybrids developed by crossing cultivated eggplant with *Solanum incanum*. Mangino *et al.* (2020) evaluated the performance of 16 introgression lines (ILs) containing chromosomal segments of *S. incanum* and reported improved vigour in the ILs but exhibited low value of various fruit related parameters. Availability of

large number of accessions (167) of *S. incanum* (Taher *et al.*, 2017) offers to select the best performing one for distant hybridization.

Inter and intraspecific hybridization transfers desired genes from one species to the other or same species which play an important role to improve the crop yield and disease resistance (Ghani *et al.*, 2020). Interspecific hybridization is an important approach in plant breeding to incorporate useful genes to crop improvement (Sekara *et al.*, 2007). The wild species of eggplant are a rich source of genes for resistance to diseases or insects. For example, bacterial wilt resistance in *S. aethiopicum*. (Collonnier *et al.*, 2001); resistance to little leaf, shoot and fruit borer in *S. indicum* (Bahgat *et al.*, 2008); Verticillium and Fusarium wilt resistance in *S. incanum* (Prohens *et al.*, 2013). However, in interspecific hybridization, the production of hybrid seed is greatly hampered due to certain fertilization barriers. The crossability and hybridization studies of different *S. melongena* accessions with its related species have been carried out with inconsistent results (Behera & Singh, 2002). Crossability of *S. melongena* with *S. incanum* has also been reported (Kumar *et al.*, 2020). The breeding materials developed through this program will help in further improvement of eggplant particularly in resistance breeding, but the fruit weight is reduced, particularly in *S. incanum* (Kumar *et al.*, 2020).

The identification of hybrids relies on both morphological traits and molecular markers. Different molecular markers, including RAPD, AFLPs, and SSR have been used in plant genetic analysis. In the present study, a marker system called sequence related amplified polymorphism (SRAP) was chosen because it is simple, reproducible and comparatively less expensive than other types of markers (Li & Quiros, 2001). Moreover, SRAP is also more informative than RAPD, ISSR, SSR, AFLP (Budak *et al.*, 2004). With respect to true hybrid, SRAPs have provided a reliable tool for the estimation of the degree of genetic relatedness among number of species in the plant kingdom

(Li & Quiros, 2001). Thus, SRAP markers are commonly used as they are rapid, simple, and cost effective to access the genetic purity of interspecific and intraspecific hybrids.

In the present study, we reported the successful interspecific hybridization of eggplant and its wild relative *S. incanum*. We tried to describe the characteristics of intraspecific and interspecific hybrids between *S. melongena* x *S. melongena* and *S. melongena* x *S. incanum* and found positive gain in fruit size and fruit yield per plant compared to the parents. Given the high quality (phenolic contents) of *S. incanum*, and tolerance to drought and resistance to a few diseases (Prohens *et al.*, 2013; Mangino *et al.*, 2020) as well as suitability as rootstock (Gisbert *et al.*, 2011), these materials may be of great interest for developing a new generation of eggplant varieties adapted to climate change and tolerance to drought and heat stress.

MATERIAL AND METHODS

Plant materials

A two-year experiment was conducted at Vegetable Research Farm, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan [30-31.5°N, 73-74°E, 184 m altitude (604 ft.)]. The three accessions of *S. melongena* and one *S. incanum* were collected from Asian Vegetable Research and Development Center (AVRDC). These germplasms were collected due to their different characters: *S. melongena* (MEE, VI042556) has spiny leaves and oblong purple fruit, *S. melongena* (MEP, VI041674) has white slightly curved long and purple fruit, *S. melongena* (MEB, VI042569) has snake shaped long purple fruit and *S. incanum* (INC, VI048616) a wild species has round shape and small green fruit.

Morphological parameters

Different morphological parameters were observed i.e. leaf length, leaf width, plant height, fruit length and fruit diameter. Leaf dimensions were recorded by taking measurements of three mature leaves from each plant at the vegetative phase. Plant height was

measured after flowering while fruit length and fruit diameter were obtained from mature fruit. These parameters were taken by using meter scale. Fruits were harvested at maturity and yield per plant was determined.

Pollen viability

The mature flower buds were bagged with butter paper bag one day before anthesis for the pollen viability assessment. Three to four flowers were collected of each wild and cultivated species at 8 a.m. on the day of anthesis. Fresh pollen from anthers of each flower of parents and F_1 were shattered on microscope glass slide and stained with 1% (w/v) acetocarmine. After drying of acetocarmine, slides were examined under microscope (400 X) using the method described by Alexander (1969).

SRAP (Sequence related amplified polymorphism) analysis

Eggplant six SRAP primers were selected from sequence previously reported (Li & Quiros, 2001). For this purpose, these six pair of primers were used Forward primer (Table 1). A modified version of the CTAB method was used to extract genomic DNA. PCR amplification was performed under the following conditions: 5 min of denaturing at 94°C, and 5 cycles of three steps, including 1 min of denaturing at 94°C, 1 min of annealing at 35°C and 1 min of elongation at 72°C. This was followed by 35 cycles of 94°C/45 s, 50°C/45 s, 72°C/1 min, 1 cycle of 72°C for 10 min at 4°C. Each PCR product (6 µL) was resolved in 6% denaturing poly acryl-amide gel

electrophoresis (PAGE) at 1500 W for 2.5 h. The amplified products were visualised by simplified silver staining. In order to obtain reproducible and clear banding patterns, each amplification was repeated three times, and only bands showing consistent amplifications were scored.

Statistical analysis

The statistical analyses were carried out by using Statistix 8.1 software. Standard deviation (SD) was also calculated. Complete randomize block design (RCBD) was used and also for the pair-wise comparisons least significant difference (LSD) test was used with 95% ($p < 0.05$) confidence.

RESULTS AND DISCUSSION

Table 1. List of eggplant SRAP markers. Faisalabad, University of Agriculture, 2019.

Pair no.	Forward primers	Reverse primers
1	me1, 5'-TGAGTCCAAACCGGATA-3'	em2, 5'-GACTGCGTACGAATTTGC-3'
2	me3, 5'-TGAGTCCAAACCGGAAT-3'	em4, 5'-GACTGCGTACGAATTTGA-3'
3	me1, 5'-TGAGTCCAAACCGGATA-3'	em1, 5'-GACTGCGTACGAATTAAT-3'
4	me2, 5'-TGAGTCCAAACCGGAGC-3'	em2, 5'-GACTGCGTACGAATTTGC-3'
5	me1, 5'-TGAGTCCAAACCGGATA-3'	em4, 5'-GACTGCGTACGAATTTGA-3'
6	me2, 5'-TGAGTCCAAACCGGAGC-3'	em4, 5'-GACTGCGTACGAATTTGA-3'

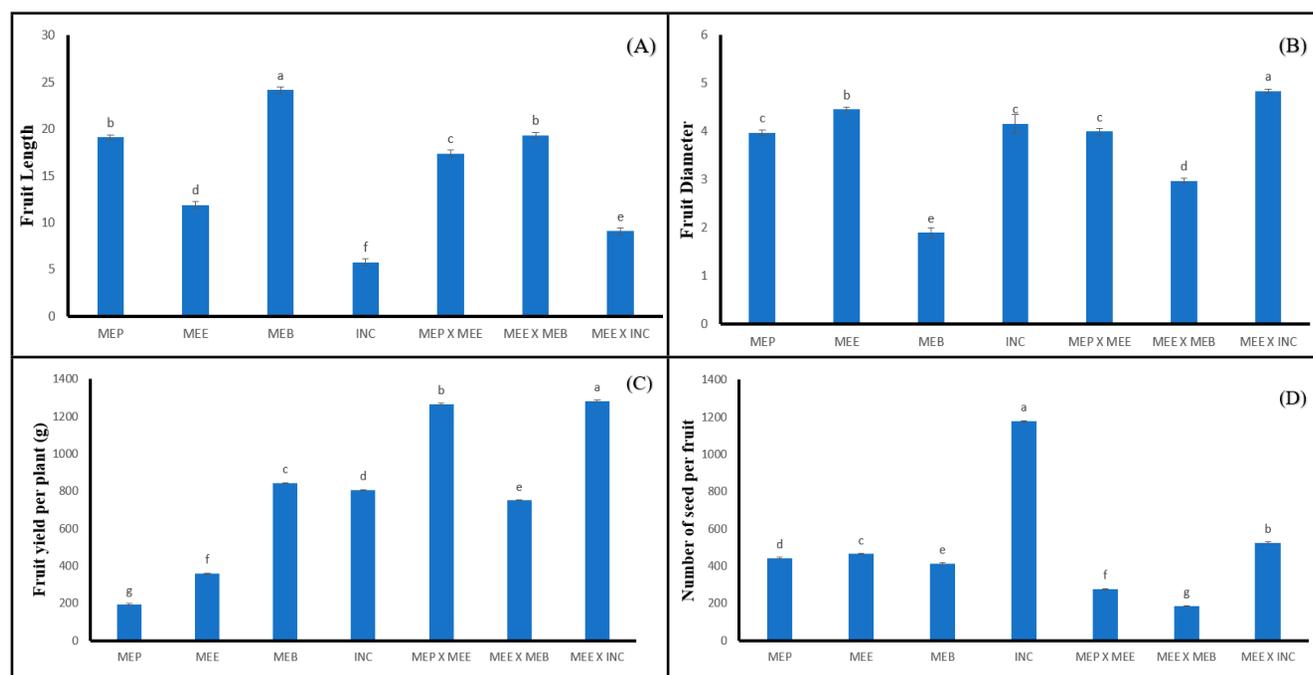


Figure 1. A) Variations in fruit length (cm); B) fruit diameter (cm); C) fruit yield per plant (g) and D) number of seeds per fruit among parents and their hybrids. Parents: MEP, MEE, MEB, INC and hybrids: MEPxMEE, MEExMEB, MEExINC. Faisalabad, University of Agriculture, 2019.

Inter and intraspecific crossing

Intraspecific hybrids were produced by using different cross combinations (Table 2). Highest hybridization resemblance was observed in MEE×MEB with a 14.81% successful cross ratio than other combinations. On the other hand, different cross combinations with *S. incanum* were also

performed to produce F₁ interspecific hybrids. In this cross-success ratio 6.12% was observed in MEE×INC combination, but its reciprocal was unsuccessful. The MEE×INC hybrid had the maximum number of seeds per fruit (Table 2).

Morphological observation

The crossability among the *S.*

melongena genotypes with their wild relatives showed wide range of variations in morphological characters. The interspecific hybrids were intermediate in most of the parental characteristics. This is a common phenomenon in interspecific hybrids in eggplant with wild relatives (Prohens *et al.*, 2013; Kaushik *et al.*, 2016). The hybrid F₁ plants were evaluated by visual morphological observation of parents and their hybrid plants. The observations pertaining to the following characters were recorded on three randomly obtained competitive plants from each treatment (Table 3). At seedling stage F₁ hybrids were no significant different from parents. To produce hybrids, different cross combinations were performed between MEP, MEB, MEE and INC (Table 3). Cotyledons length results indicated that the parent MEP has maximum length (3.22 cm) while minimum length was recorded in MEB (1.46 cm) compared to hybrids. However, the MEP×MEB hybrid had the highest cotyledon width (0.51 cm) while MEE had lowest cotyledon width compared to other parents and hybrids (Table 3). The hybrid MEP×MEB

Table 2. Total number of cross combinations of inter and intraspecific hybridization of eggplant. Faisalabad, University of Agriculture, 2019.

Combination	No. of crosses	No. of fruit setting	Successful cross (%)	No. of seeds/ fruit
MEE × MEB	54	8	14.81	190
MEE × INC	49	3	6.12	550
MEP × MEE	50	7	14.00	250
MEP × MEB	34	0	0.00	0.00
MEP × INC	58	0	0.00	0.00
MEB × MEP	41	0	0.00	0.00
MEB × MEE	29	0	0.00	0.00
MEB × INC	58	0	0.00	0.00
INC × MEP	70	0	0.00	0.00
INC × MEB	61	0	0.00	0.00
INC × MEE	62	0	0.00	0.00
MEE × MEP	66	0	0.00	0.00

Table 3. Morphological observations along with pollen viability test of parents and their hybrids. Parents; MEP, MEE, MEB, INC and hybrids; MEP×MEE, MEE×MEB, MEE×INC. Faisalabad, University of Agriculture, 2019.

Characteristics	MEP	MEE	MEB	INC	MEP x MEE	MEE x MEB	MEE x INC
Cotyledon length (cm)	3.22a	2.56bc	1.46d	3ab	2.62e	2.7f	2.38c
Cotyledon width (cm)	0.5a	0.2d	0.33bc	0.394bc	0.434ab	0.51a	0.436ab
Plant height (cm)	51.48c	51.4c	43.64d	43.88d	61.88b	66.26a	62.42b
Plant Growth habit	intermediate	intermediate	upright	upright	upright	prostrate	upright
Stem diameter (cm)	1.72c	2.89a	2.1bc	1.61d	3.12ab	1.64b	2.26bc
Leaf length (cm)	20.2c	23.1b	21.1c	22.6b	27.6a	23.54b	28.1a
Leaf width (cm)	11.86c	10.5b	14.5a	11.258ab	11.726d	14.6abc	15.1ab
Leaf petiole length (cm)	4.748e	6.694cd	6.324d	7.164bc	8.756a	7.176bc	7.66b
Leaf blade color	green	light green	dark green	light green	dark green	green	green
Days to flowering	61.6e	94.4b	106a	63.4e	80.2c	78cd	76.4d
Corolla color	pale violet	white	bluish violet	light violet	light violet	pale violet	light violet
Sepal length (cm)	2.15a	1.754c	2.164a	1.862b	1.756c	1.664d	1.656d
Petal length (cm)	2.752b	2.862a	2.452d	2.866a	2.806b	2.578c	2.726c
*Fertile	70.1 (18.8 ^{cd})	40.9 (16.2 ^d)	42.4(19.2 ^{ab})	42.6(21.6 ^{bc})	63.4(26.4 ^b)	38.9(17.2 ^c)	61.1 (32.4 ^a)
*Semi fertile	35.07 (9.4 ^e)	59.09(23.4b ^e)	65.04(29.4 ^a)	60.8(30.8 ^a)	36.5(15.2 ^d)	61.08(27 ^c)	(20.6 ^e)

*The category was based on the assessment of Devi *et al.* (2015). Means with the same letter are not significantly different.

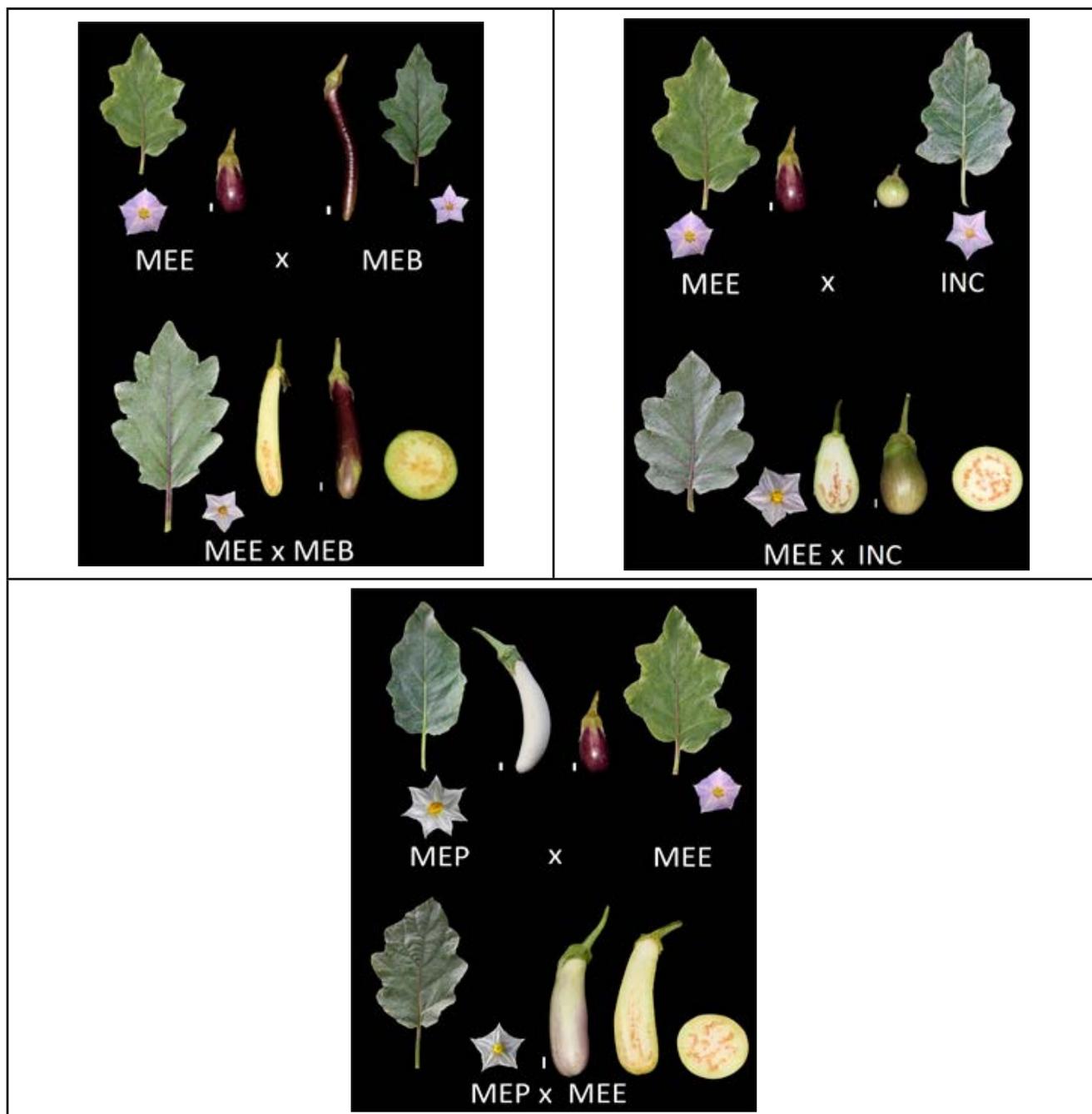


Figure: 2a. The inter and intraspecific hybridization have shown the comparison between the hybrid leaves, flowers and fruits with their parents. Parents: MEP, MEE, MEB, INC and hybrids; MEPxMEE. Faisalabad, University of Agriculture, 2019.

gained maximum plant height (66.26 cm), while the parent MEB exhibited minimum height about 43.64 cm than the other parents and hybrids (Table 3). The maximum stem diameter was observed in hybrid MEPxMEE other than hybrids and parents (Table 3). Leaves are main sites for photosynthetic reaction and play a key role in fruit size and yield (Shakoor *et al.*, 2010). In interspecific crosses, wild parent INC

showed intermediate length, width and leaf petiole length. MEExINC hybrid had the highest leaf length (28.1 cm) and leaf width (15.1 cm) compared to parents and hybrids. The hybrid MEPxMEE showed maximum petiole length (8.75 cm), while the parent MEP minimum petiole length (4.748 cm) than the other parents and hybrids. However, the flower is a most critical reproductive part of the plant which plays key role in

yield of plants. Moreover, Haydar *et al.* (2007) also found that the days taken for flower setting is directly related to the fruit yield in tomato. Parent MEP has minimum days to flowering about 61.6 days than the other parents and hybrids. The maximum sepal length was measured in parent MEB (2.16 cm) and minimum length was 1.66 cm in hybrid MEExINC (Table 3). Highest petal length was measured in

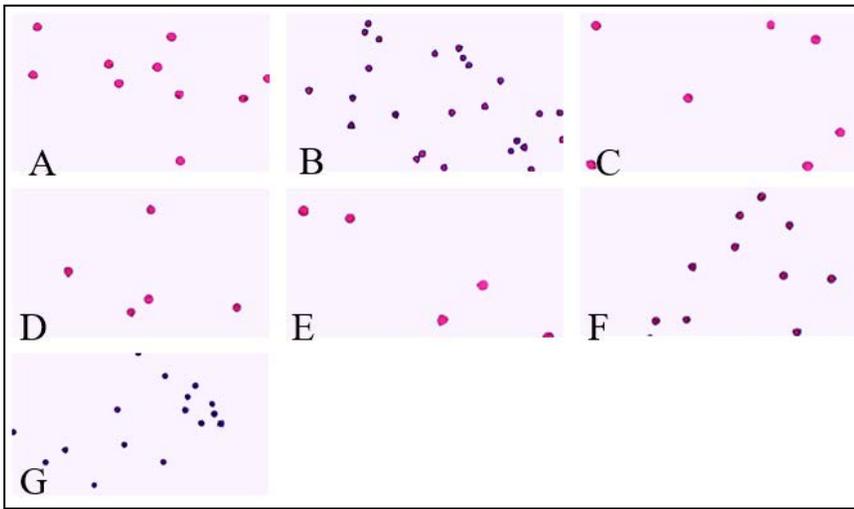


Figure 2b. Pollen fertility of parents, (A) INC (semi-fertile), (B) MEP (fertile), (C) MEE (semi-fertile), (D) MEB (semi-fertile), and pollen fertility of hybrids (E) MEExMEB, (semi-fertile) (F) MEExINC (fertile), (G) MEPxMEE (fertile). Faisalabad, University of Agriculture, 2019.

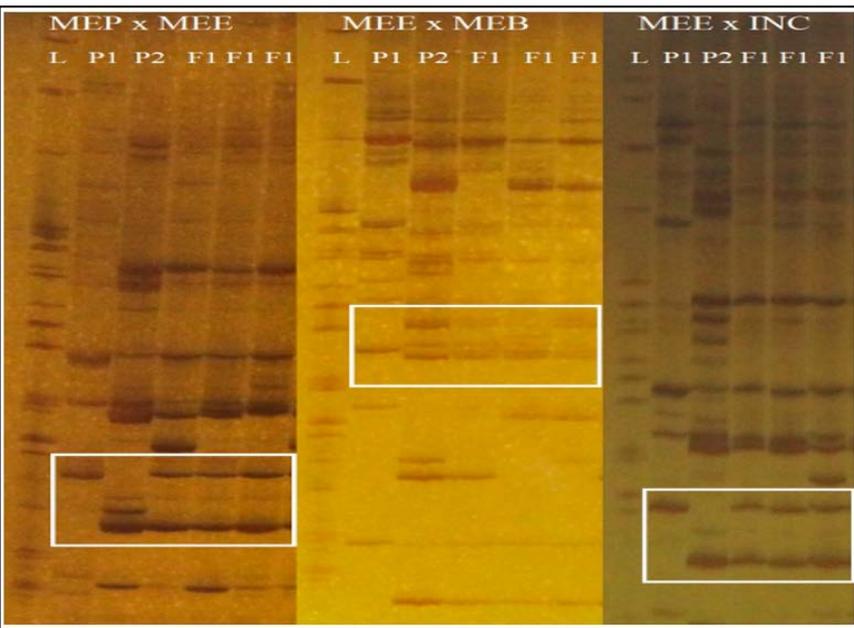


Figure 2c. Analysis on SRAP result of inter and intraspecific (F1) hybrid and their parent lines using markers. L: leader; P: parents. F₁ hybrids. Parents: MEP, MEE, MEB, INC and hybrids: MEPxMEE, MEExMEB, MEExINC. Faisalabad, University of Agriculture, 2019.

INC (2.87 cm); on the other hand, the lowest petal length was observed in hybrid MEExMEB (2.57 cm) (Table 3). Stamen length results indicated that maximum length was 1.54 cm in hybrid MEExINC and minimum length was 1.36 cm in INC (Table 3). The fruit length was observed maximum (24.16 cm) in MEB parents compared to other parents and hybrids (Figure 1a). But the fruit diameter was maximum (4.84 cm)

in MEExINC hybrid other than parents and hybrids (Figure 1b). The parent INC showed maximum pedicle length (7 cm), while the parent MEB possessed minimum pedicle length (3.18 cm) than the other parents and hybrids (Table 2). The highest fruit pedicle thickness was recorded in parent INC (1.16 cm) while minimum fruit pedicle thickness (0.6 cm) was recorded in MEE. Fruit yield was measured on per plant basis

and the results indicated that the hybrid MEExINC had highest fruit yield 1283.8 g while, on the other hand, parent MEP had lowest fruit yield per plant (194.4 g) among all parents and hybrids (Figure 1c). Interspecific hybridization between cultivated eggplant and its wild relatives is an important way of deploying desirable genes for eggplant improvement (Liu *et al.*, 2015). In this study, the MEE x INC hybrid was successfully obtained by crossing cultivated eggplant and *S. incanum*. However, when *S. incanum* was used as female, no fruit setting was observed, whereas only cross combinations of cultivated eggplant and *S. incanum* showed fruit setting when *S. incanum* was used as male. Most of the attempts to produce interspecific hybrids have resulted in sterility between the eggplant *S. melongena* and its wild relatives (Nasrallah & Hopp, 1963). Latest literature described that the hybrids of *S. melongena* x *S. incanum* and *S. incanum* x *S. melongena* were fertile and produced seeds (Kaushik *et al.*, 2016). The INC parent had highest number of seeds per fruit (1177.2) and the lowest numbers of seeds (185) was recorded in MEExMEB as compared to parents and hybrids (Figure 1d). The maximum number of seeds per fruit was also found in *S. melongena* x *S. incanum* as compared to parents and other hybrids (Figure 2a). Higher number of seeds per fruit was also reported in a cross between *S. melongena* x *S. incanum* (Plazas *et al.*, 2016). But the other study showed that the number of seeds per fruit was low in *S. melongena* and their wild relatives (Devi *et al.*, 2015).

Pollen viability estimation

Pollen viability of hybrids and their parents was found different. The INC, MEE and MEB were semi-fertile and showed pink color of pollen grains. The pollen grains of MEP gave dark red color and were highly fertile, i.e. 70.1% pollens were fertile, while 63.4% pollen were fertile in hybrid MEP x MEE. On the other hand, the least pollen viability (38.9%) was observed in MEE x MEB. However, the maximum (65.04%) semi-fertile pollens were observed in MEB parent and minimum semi-fertility

was observed in 35.07%. Pollen grain of MEE_xMEB gave pink color that indicated semi-fertile pollen while pollens of MEE_xINC and MEP_xMEE were dark red colored indicating high fertility and so they can be used in further breeding program (Figure 2b). A previous study also reported wide range of variation for pollen viability in *S. melongena*, wild relatives and their hybrid progenies (Devi *et al.*, 2015). Almost all the pollen was aborted and empty, only 1.2% appearing normal, and pollen fertility was also high with 77.7% stainable pollen which was also larger than that of the parents.

Molecular analysis by SRAP markers

SRAP analysis was also performed to confirm the hybridity of MEP_xMEE, MEE_xINC and MEE_xMEB. For confirmation of hybridity, all plants were analyzed by SRAP markers, and the results showed that they had specific bands from the two parents (Figure 2c). Thus, all plants were true hybrids. Similarly, all hybrids were found to be true hybrids between ‘Clementine’ mandarin and K1 blood orange (Oruç & Dalkiliç, 2017). Mishra *et al.* (2011) demonstrated the effectiveness of SRAP marker in genetic analysis and hybrid identification of intraspecific *C. arabica* coffee hybrids.

These results suggest that genotypic difference can result in variable results of heterosis observed for positive gain in fruit diameter and yield of *S. melongena* x *S. incanum*. In this regard, this material will be of great interest for the genetic improvement of eggplant; it may have a tremendous potential to increase tolerance to abiotic stresses, such as to drought and heat, as well as increased the nutrient values and herbal values which will be helpful in improving the human health. Finally, we hope that this study opens the way for the incorporation of the *S. incanum* in new World gene pool for eggplant breeding, ultimately contributing to the development of a new generation of plants adapted to climate change and with improved nutritional, herbal, and diseases and pest resistance properties.

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