Assessment of Polycross Hybrids of Mulberry for Fruit and Seed Traits

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AUTHORS CONTRIBUTION

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ABSTRACT

Mulberry is a fast growing woody perennial tree that maintains high heterozygosity due to the out-breeding reproductive system. The success of breeding programme mainly depends on availability of genetic variability and selection of superior progenies. Polyclonal seed orchard is one of the sources for creating huge desirable genetic variability using natural hybridization with minimum effort for improvement of leaf yield and quality in mulberry. High bush polyclonal seed orchard was established using twenty nine parents (16 female & 13 male) at CSR&TI Berhampore. Morphological assessment of hybrid fruits and seeds were performed for two successive years (2020 & 2021). The results showed that significant differences among polycross hybrids in most of the fruit & seed traits. Fruit weight varied from 0.63g (T-13) to 2.27g (Berhampore-A); fruit length varied from 1.58cm (T-13) to 2.65cm (CSRS-1); fruit width varied from 0.86cm (Matigera-black) to 1.32cm (Berhampore-A); seeds per fruit varied from 25 (C-2038) to 47 (Berhampore-A) and test weight varied from 1.25g (China-white) to 2.20g (C-2045). The highest seed set was recorded in C-2045 (91.95%) followed by Sujanpur-5 (88.70%). Highly significant positive correlation was observed between fruit weight & fruit width (0.87); fruit weight & fruit length (0.84); achenes per fruit & seeds per fruit (0.72). A significant difference was recorded for seed germination among 16 polycross & it varied from 70 *per cent* (China-white & S-30) to 99 *per cent* (C-2038). After 60 days, germination was reduced more in Sujanpur-5 (92.5%) and Bogura-4 (90.40%). The information may be useful for the breeders to identify superior seedlings and creating heterozygous hybrid progenies in mulberry.

Keywords : Polycross hybrid of mulberry, Polyclonal seed orchard, Seed germination, Seed set, Fruits traits, Seed traits

SERICULTURE is an agro based industry and the final product of this industry is silk. The improved mulberry varieties play an important role in enhancing silk productivity and profitability of sericulture. Due to new silkworm hybrids, more number of silkworm crops per year & demanding high quality silk, superior mulberry varieties have been gaining importance. Hence, creation of desirable variation is indispensable. There are different methods to create genetic variability, bi-parental crossings or traditional breeding is most common where few parents with desired traits are considered. But, this process is time consuming & laborious. In mass or open pollinated hybrids, pollen source is unknown thus undesirable traits may occur in the OPH varieties as well as general combing ability of a variety cannot be estimated. To overcome such limitation, polycross breeding is being practiced since long time especially for obligate cross pollinators in particular those that can be propagated vegetatively (Frandsen and Frandsen, 1948). A polycross is a mating arrangement for inter pollinating a group of cultivars or clones using natural hybridization in an isolated crossing block (Klein *et al.,* 1973). Progeny from each entry have a common parent in a polycross design. Thus, half sib families are generated & these are frequently used for evaluating general combining ability. Purpose of polycross breeding is to provide equal opportunities for each entry to be crossed with other entry.

Mulberry is an extremely versatile plant that can fulfill a number of roles in small holding agricultural production. The mulberry (*Morus* sp.) leaf is the sole food source for the mulberry silkworm (*Bombyx mori* L.) and contributes 38.20 per cent towards the success of a cocoon crop (Wani *et al.,* 2018). Mulberry can be propagated through various methods *viz.,* seeds, cuttings, layering, grafting and tissue culture, etc. Mulberry can be easily propagated through cuttings, however has limitations which includes lack of variations resulting in reduced adaptability of daughter plants, restriction of raising only region specific plant varieties, reduced vigour by subsequent generations and lack of tap root system in vegatatively propagated plants with least robustness in them (Leaky, 2014). In recent years, the importance on mulberry fruits/ seeds has increased together with understanding of their nutritional capacities and breeding new potential cultivars for improving fruit yield [Ozgen *et al.,* 2009].

The sexual reproduction of mulberry through seeds is essential to generate and maintain genetic variability. The morpho-physiological traits are prominent, because they can be rapidly determined and have an important predictive value concerning plant adaptation (Sanchez *et al.,* 2015). In this sense, the internal structure of seeds, particularly embryo morphology, is a valuable piece of information for the classification of seed dormancy (Baskin and Baskin, 2007; 2014). The seed size and mass are vitally important traits in the life cycle of a plant, because they have implications in the dispersal, establishment and survival mechanisms of the species. In addition, the hydration degree of seed plays a fundamental role in their longevity and germination performance (Jimenez-Alfaro *et al*., 2016). However, other seed characteristics could also show the responses of the species to the environment; for example, the physical defense structures (testa/ endocarp) and the nutrient content in the seed reserves-embryo/ endosperm (Daws *et al.,* 2006; Montejo *et al.,* 2015). Therefore, the objective of this study was to characterize the different morpho-physiological traits of the fresh fruits and seeds of sixteen polycross hybrid progenies.

Material and Methods

The field experiment was conducted during 2018 to 2021 at Central Sericultural Research and Training Institute, Berhampore (West Bengal). Twenty nine parents (16 female and 13 male parents) were selected for establishment of polyclonal seed orchard based on the available pedigree records on flowering synchronization, sex expression, ploidy level, *per se* performance on leaf quality & productivity parameters, genetic diversity and combining ability studies (Table 1). The land for the establishment of seed orchard was selected far away from general mulberry plantation and surrounded by S-1635 plantation which is triploid variety bears no fertile pollen. Saplings were raised from mature cuttings of selected parents. After 10 months, the saplings were transplanted at a spacing of 5’×5’ in such a way that each male parent is surrounded by different female parents. In this manner, all males and female parents were repeated many times to ensure sufficient plants to generate sufficient quantity of hybrid seeds. The plants were trained as high bush plantation and pruning was carried out once a year during July-August (Fig.1). Recommended package of practices for mulberry cultivation under irrigated condition was followed to raise healthy plantation (Ray *et al.,* 1973).

Collection of Hybrid Fruits and Extraction of Seeds : After natural pollination during flowering season in 2020 & 2021, polyclonal hybrid (PCH) fruits were harvested from individual female parents of seed orchard. Fruits of each mulberry genotypes were soaked in water separately for 48 hours and seeds were extracted by water soaking method (Barbour *et al.,* 2008). Harvested seeds were air dried in a

TABLE 1

Parents utilized to establish Polyclonal Seed Orchard

Parents	Acc No. / Parentage
Female Parents	
Kosen	ME-0066
China White	ME-0042
C-2038	CF1(10) × C-763
C-2045	MHP × CF1(13)
C-2036	MHP × CF1(23)
C-2060	KOP × V1
S-30	MI-0046
Kajli OP	OPH of Kajli
Phillipines	ME-0011
CSRS-1	MI-0084
Berhampore-A	MI-0054
Sujanpur-5	MI-0017
M. Black	MI-0300
Bogura-4	ME-0097
T-13	Elite clone
Kajli	MI-0068
Male Parents	
MS-1	MI - 0054
MS-7	MI - 0001
Mandalaya	ME-0043
Sultanpur-5	MI-0098
Bisanpur-10	MI - 0092
White Badan	MI - 0300
Almora	MI-0141
Molai	ME -0003
Monali	MI-0131
Jodhapur	MI - 0248
M. multicaulis	ME -0006
C-776	*M. nigra* × *M. multicaulis*
CT-44	*M.indica* HP × $CF_1$12

room for two days and stored in desiccators filled with silica gel for maintaining viability for longer time.

Characterization of Hybrids Fruits and Seeds : Average performance (Two consecutive years) of fruit length, fruit width, fruit weight, No. of seeds/fruit, No. of achenes/fruit, test weight (weight of 1000 seeds) and seed viability & germination was recorded in PCHs of all the crosses following the standard procedures (Dwivedi, 1990). Seed viability and germination of hybrids were recorded from one hundred seeds in four replications. Seed viability was measured by Tetrazolium method (Dandin, *et al.,* 1991). Water soaked seeds were washed and cut in to two equal halves then were dipped in 1 per cent 2,3,5-triphenyl tetrazolium chloride solution for 24 hours. Pink colour seeds out of total seeds tested were used to calculate seed viability percentage. Top paper method was used for measuring seed germination (%) using wet 3 layers of blotting paper in petriplates (Rao *et al.,* 2006). Seed viability and germination was observed after 0, 30 and 60 days after seed extraction.

RESULTS AND DISCUSSION

The genetic improvement of mulberry depends on the availability of genetic variability and selection of suitable genotypes from breeding population. Mulberry is highly heterozygous species and success of breeding programme largely depends on generation of larger amount of desirable variability and selection of superior progenies. The greater amount of variability provides a scope for selecting desirable recombinant for more number of traits. Polycross mating design has been served as a source of enormous desirable genetic variability for improvement of yield and quality in mulberry. Twenty nine parents were utilized to establish a polyclonal seed orchard during 2018-19 (Table 1 & Plate 1). The seed orchard was trained to high bush plantation with recommended cultural practices. The established seed orchard was isolated from other genetic sources ensured production of true hybrids progenies. Polycross hybrid fruits from each parent were collected during March-April months after natural pollination during the year 2020 & 2021. Further, polycross fruits & seeds from 16 parents were characterized and the results revealed presence of enormous genetic variations for characteristics studied.

Morphological characters of PCH fruits & seeds were recorded for two consecutive years (2020 & 2021). Among 16 polycrosses, Berhampore-A cross

Plate 1 : Established polyclonal seed orchard bearing flowers, fruits and seeds

TABLE 2

Pooled average performance of fruit and seed parameters of Polycross hybrids

Polycross progeny derived from	Fruit length (cm)	Fruit width (cm)	Fruit Weight (g)	Seeds / fruits (No.)	Test Weight (g)	Achenes/ fruit (No.)	Seed set (%)
Kosen	1.59	0.95	0.83	35.80	1.39	47.60	75.21
China White	2.10	1.06	1.26	40.30	1.25	57.60	69.97
C-2038	2.61	1.05	1.43	25.00	1.76	40.00	62.50
C-2045	2.28	1.17	1.52	40.00	2.20	43.50	91.95
C-2036	2.08	1.09	1.78	44.25	1.98	54.00	81.94
C-2060	2.45	0.95	1.67	29.00	1.79	48.60	59.67
S-30	2.35	1.05	1.39	43.20	1.38	57.00	75.79
Kajli OP	2.36	1.25	2.18	41.00	1.32	56.00	73.21
Phillipines	1.69	0.98	0.79	28.00	1.87	35.60	78.65
CSRS-1	2.65	1.11	1.98	39.80	1.56	68.40	58.19
Berhampore-A	2.56	1.32	2.27	47.00	1.43	62.20	75.56
Sujanpur-5	2.19	0.94	0.94	42.40	1.42	47.80	88.70
Matigera Black	1.66	0.86	0.74	37.50	1.55	46.20	81.17
Bogura-4	1.69	0.99	0.78	34.00	1.83	48.60	69.96
T-13	1.58	0.88	0.63	31.25	1.36	40.30	77.54
Kajli	1.93	0.87	0.88	32.20	1.46	46.20	69.70
CD (P 0.05)	0.20	0.07	0.29	3.42	0.15	4.63	5.02

Plate 2 : Morphological variability observed among the seeds of different polycross hybrids

recorded highest fruit weight followed by Kajili OP & CSRS-1. Higher fruit weight and yield among the mulberry genotypes was also reported by Ercisli 2004; Orhan & Ercisli, 2010. Seed size is an important physical indicator of seed quality that affects vegetative growth. The polycross C-2045, C-2036 & Phillipines had higher test weight and significant genetic variation among the varieties studied. The presence of large genetic variation for seed size and higher performance in seedlings derived from larger seed was reported by Ambika *et al.,* 2014. Number of seeds per fruit is important seed yield parameters and it was highest in Berhampore-A followed by C-2036 & S-30. Seed set percentage varied among polycrosses studied and C-2045, Sujanpur-5 & C-2036 recorded more than 80 per cent seed set (Table 2 & Plate 2).

Higher seed viability was noticed among 16 polycrosses on the day of extraction. Higher viability was maintained in seeds preserved under desiccators even after two months after extraction (Plate 3). The seed germination percentage varied from 70 per cent (China white & S-30) to 99 per cent (C-2038) on the day of extraction among the 16 polycrosses studied. After 60 days, germination percentage of polycross seeds varied between 6 per cent (Sujanpur-5) to 71 per cent (Berhampore-

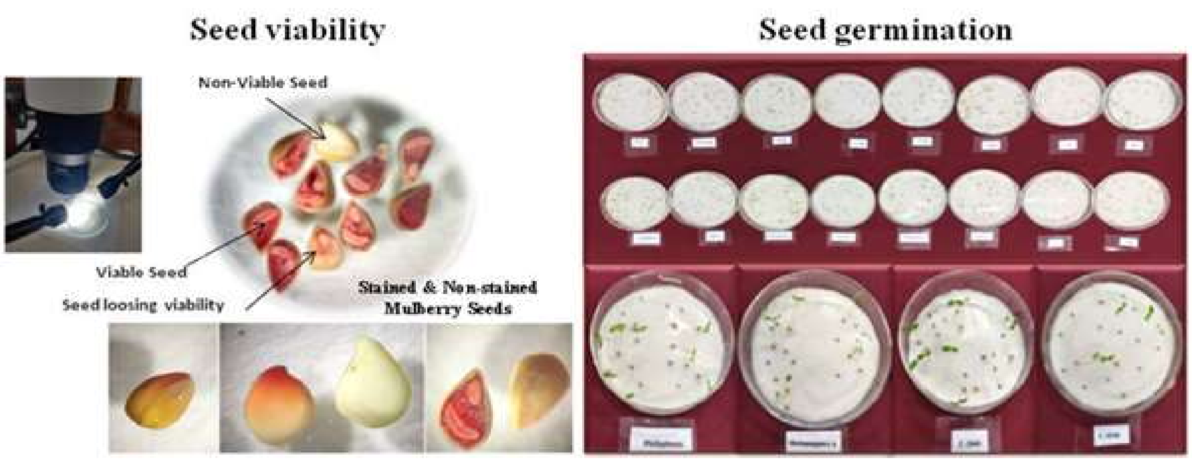

Plate 3 : Viability and germination of seeds of polycross hybrid progenie

TABLE 3
Seed viability and germination in polycross hybrids

Polycross progeny derived from	Seed Viability (%)			Seed Germination (%)		
	0 days	30 days	60 days	0 days	30 days	60 days
Kosen	100	95	98	85	60	22
China White	99	95	96	70	48	9
C-2038	99	99	97	99	73	16
C-2045	98	94	95	80	51	11
C-2036	99	96	96	80	49	13
C-2060	100	99	100	90	74	40
S-30	99	93	94	70	49	16
Kajili OP	98	96	96	91	61	31
Phillipines	98	94	93	94	80	55
CSRS-1	98	97	98	81	70	18
Berhampore-A	100	97	97	96	80	71
Sujanpur-5	99	98	99	80	50	6
Matigera Black	99	97	97	92	70	34
Bogura-4	99	95	96	83	56	8
T-13	99	98	99	91	53	13
Kajili	97	94	97	89	78	61
CD (P 0.05)	0.44	1.00	0.98	6.75	13.21	10.74
CV (%)	1.91	2.92	2.86	5.53	14.79	28.41

A) indicating decrease in germination with increase in storage period (Table 3). Similar results were reported in mulberry by Gunduz *et al.,* 2019 (20% - 30% in *Morus bombycis*) and Song *et al*., 2016 (9.3% - 66.5% in different mulberry species).

Reduction in seed germination was calculated after 30 as well as 60 days after extraction. Highest reduction in seed germination was observed in Sujanpur-5 (92.5%) followed by Bogura-4 (90.40) & China white (87.1%) at 60 days. However, at 30

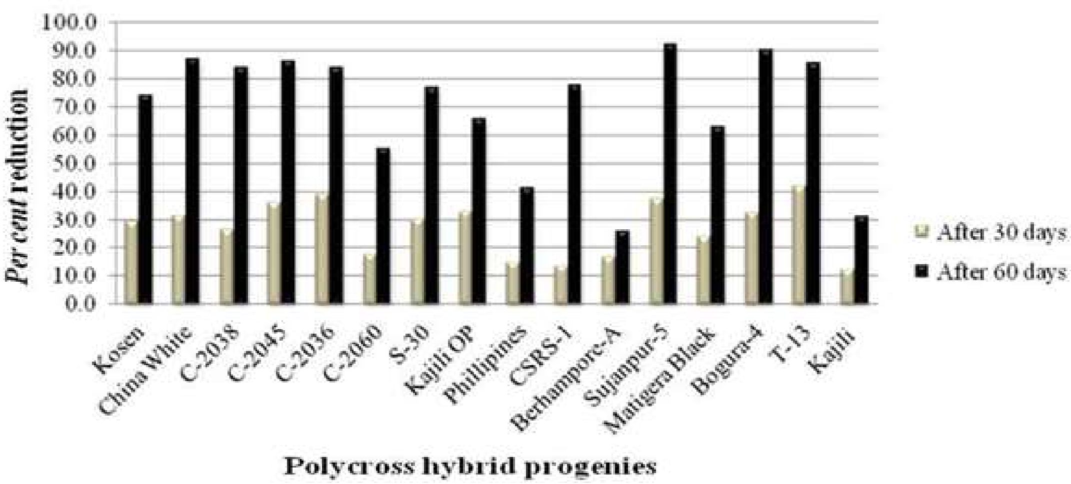

Fig.1 : Per cent reduction of seed germination in polycross hybrid progenies during storage

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Table 4

Association of fruit and seed characters of mulberry

Parameters	Fruit length (cm)	Fruit width (cm)	Fruit weight (cm)	Seeds/ fruit (No)	Test weight (g)	Achenes/ fruit (No)	Seed Set (%)
Fruit length (cm)	1						
Fruit width (cm)	0.66 *	1					
Fruit weight (g)	0.84 *	0.87 *	1				
Seeds/ fruit (No)	0.27	0.56 *	0.49	1			
Test weight (g)	0.05	0.09	0.05	-0.23	1		
Achenes/ fruit (No)	0.55 *	0.57 *	0.69 *	0.72 *	-0.37	1	
Seed Set (%)	-0.36	0.01	-0.25	0.42	0.20	-0.31	1

* Significant at the 0.05 level

days after seed extraction reduction percentage ranged from 12.4 per cent to 41.8 per cent indicating, that mulberry seeds should be sown within a month of harvesting (Fig.1).

In any breeding program of complex characters such as yield for which direct selection is not effective, it become essential to measure the contribution of each of the component variable. The association between two characters that can be directly observed is the correlation of phenotypic value or the phenotypic correlation (Falconer & Mackay, 1996). A significant positive phenotypic association was observed between fruit length and fruit width, fruit weight & achenes per fruit. Similarly, fruit width had significantly positive correlation with fruit weight, seeds per fruit and achenes per fruit. In addition, seed set was found to be in positive association with seeds per fruit & test weight and negative association with fruit length, fruit weight & achenes per fruit (Table 4), which is supported by the findings of earlier workers (Dandin *et al.,* 1987 and Vijayan *et al.,* 1997).

Polycross hybrid progenies were developed using twenty nine genetically diverse parents. Morphological characterization of polycross hybrids revealed huge genetic variability present among sixteen half sib families. Seed viability & germination of different polycross hybrids had a wide range and gradually reduced with the increase in storage period *i.e.* after two months of seed extraction. Some of the characteristics of fruits & seeds showed positive association among themselves. The information with respect to fruit and seed traits will support the breeders for intra and inter specific hybridization with suitable parents for developing large number of progenies with desired economic characters to select the best one and in turn benefit the sericulture farming community and the industry as a whole in the country.

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