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J Jamatia

Division of Vegetable Science,
ICAR- Indian Agricultural,
Research Institute, New Delhi,
India

H Choudhary

Advanced Centre for Plant
Virology, Division of Plant
Pathology, ICAR-Indian,
Agricultural Research Institute,
New Delhi, India

B Basavaraj

Advanced Centre for Plant
Virology, Division of Plant
Pathology, ICAR-Indian,
Agricultural Research Institute,
New Delhi, India

Correspondence**J Jamatia**

Division of Vegetable Science,
ICAR- Indian Agricultural,
Research Institute, New Delhi,
India

Morphological studies on interspecific hybrids between *Citrullus lanatus* L. and its wild species

J Jamatia, H Choudhary and B Basavaraj

Abstract

The present investigation was carried out to obtain interspecific crosses in genus *Citrullus* involving two wild relatives of *C. lanatus* var. *citroides* (DWM 32 & DWM 35) (diploid, $2n = 22$), a wild perennial *C. colocynthis* and Sugar Baby (diploid, $2n = 22$). The genotypes DWM 32, DWM 35 and DWM 210 used as male parents and Sugar Baby as female parent. The interspecific crosses yield three hybrids. The F_1 progeny resembles mostly with *C. colocynthis* for many characters like perennial growth habit, leaf colour and leaf lobing, flower colour, and bitter spongy pulp of fruit and bitterness of flesh as well as seeds. Some characters like fruit shape and rind pattern on ripening, shape and colour of seeds resembled to that of cultivated type Sugar Baby. The fruit size, seed size and flesh colour were found to be somewhat intermediate between *C. colocynthis*, *C. lanatus* var. *citroides* and *C. lanatus* var. *lanatus*. There is variation in the expression of three different F_1 progenies from wild relatives in response to WBNV. The main aim is to transfer resistance traits from wild into cultivated for future crop improvement studies.

Keywords: *C. colocynthis*, *C. lanatus* var. *citroides*, interspecific hybridization, Watermelon bud necrosis virus (WBNV)

Introduction

The genus *Citrullus* belonging to *Cucurbitaceae*, comprises two species namely *C. colocynthis* (L.) Schrad. and *C. vulgaris* (L.) Schrad. and is well represented in the semi arid and arid region of western India (Singh & Yadav, 1977) ^[1]. Watermelon occupies an area of 3.6 million ha and its production is more than 98 million tonnes (FAOSTAT, 2014) ^[1]. It is cultivated in about 84,000 ha area with a production of 20.49 lakh tonnes in India (NHB, 2015) ^[6]. Watermelon has been certified as a heart-healthy food by the American Heart Association because it is low in calories, sodium, cholesterol and fat. *Citrullus colocynthis* is considered as putative ancestral or progenitor species of watermelon which is generally found to be grown in India in north western plain in fallow lands / field and it is drought hardy and a wide diversity is found in Rajasthan and Gujarat. Tumba, *C. colocynthis* Schrad. an unexploited perennial creeper growing wild in hot Indian arid zone has attained new heights in recent past because of its multifarious uses. Another wild species *Citrullus lanatus* var. *citroides* Mansf. Is also known as the citron or preserving melon. Its rind is used to make pickles, and fruits are fed to livestock (Dane and Liu, 2007) ^[2]. Tumba is an oil rich plant ^[4, 5]. Both *C. colocynthis* and *C. lanatus* var. *citroides* have good amount of variability of several attributes. Artificial crossing experiments between races of *C. colocynthis* and *C. lanatus* have been carried out by several investigators (Singh A. K., 1977; Sain *et al.*, 2002) ^[8, 7].

Watermelon crop is known to be infected by many diseases causing loss in yield. Besides, fungal and bacterial infections, it is affected by number of viruses. Among all the diseases, Watermelon Bud Necrosis Virus (WBNV) is another *Tospovirus*, (family *Bunyaviridae*) which has emerged as a serious pathogen (Jain *et al.*, 1998; Mandal *et al.*, 2003) ^[4] causing up to 100% crop loss in watermelon in India. Phenotyping of watermelon genotypes for WBNV resistance was attempted during 2010-2011 and 2011-2012. *C. colocynthis* was found to be immune in earlier study by Holker, S. K. (2015). However *C. colocynthis* has been evaluated under field condition in Delhi conditions only by Holker, S. K. (2015). Inter specific hybridization is important for introgression of resistance genes from wild species to cultivated varieties of watermelon. The present investigation was aim to transfer desirable traits from wild species into cultivated and to study their behavior for future crop improvement studies.

Materials and Methods

In the present investigation, popular watermelon cultivar Sugar Baby (*Citrullus lanatus* var. *lanatus*) was selected as female parent and three male parents were selected from two wild species *C. colocynthis* (DWM 210) and *Citrullus lanatus* var. *citroides* (DWM 32 & DWM 35). These two accessions *Citrullus lanatus* var. *citroides* and a *Citrullus colocynthis* genotype collected from Bikaner, India (Table 1). The interspecific hybridization was carried out at the Research Farm of Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi during the *Spring Summer* and *kharif* seasons of 2015. The nursery was grown in polythene bags in polyhouse during January and 30 days old seedlings were transplanted on 18th February 2015. The evaluation of inter-specific hybrids along with parents was carried out during *kharif* (August-November) 2015. The plants were spaced at 2.5 m from row to row and 0.75 m from plant to plant within a row. Female parent Sugar Baby was

crossed with three wild genotypes to produce their F1 progeny. Healthy female flower buds, a day before anthesis were selected and bagged with butter paper. The selected male buds are covered with non-absorbent cotton to prevent from bees visit. The next day early morning the stigma remains fully receptive at the time of anthesis of female plant were dusted with pollen from two wild genotypes. After pollination, the flower bags are again bagged with butter paper to avoid contamination from foreign pollens through bees visit. Several small punched make on the butter papers to facilitate proper aeration of developing young fruits and the tag with appropriate information are tie onto the crosses to make less confusion between self and hybrids. Two days later bags were removed after proper development of the fruit. The following morphological data were recorded on several attributes based on leaf shape and size, flower colour, rind pattern, fruit shape and size, flesh colour and TSS etc.

Table 1: The species used in the investigation, its source and genotypes of genus *Citrullus*

Species	Chromosome	Genotypes	Habitats	Sources
<i>Citrullus lanatus</i> (L) Schrad	Diploid, 2n = 22	Sugar Baby	Cultivated annual	Exotic collection from USA
<i>Citrullus lanatus</i> var. <i>citroides</i> (L) Schrad	Diploid, 2n = 22	DWM 32	Wild semi perennial	Indian collection from Bikaner, Rajasthan
		DWM 35	Wild semi perennial	
<i>Citrullus colocynthis</i> (L) Schrad	Diploid, 2n = 22	DWM 210	Wild perennial	Indian collection from Bikaner, Rajasthan

The resistant lines up to 45 days were used for development of inter-specific hybrids by crossing with female parent Sugar Baby. The 3 inter-specific hybrids along with their 4 parents were evaluated during *kharif* season (August-November) in the field. Observation on bud necrosis disease (BND) incidence was recorded on 5 plants in each replications and per cent disease incidence was calculated based on the formula as given below:

Disease incidence (%) = (No. of infected plants / Total no. of plants observed) × 100

Further, the host reaction was validated based on quantification of virus concentration within the genotypes through direct antigen coating enzyme linked immuno sorbent assay (DAC-ELISA) (Clark and Bar-Joseph, 1984) at Advance Centre of Plant Virology, Division of Plant Pathology using the polyclonal antiserum available there.

Results and Discussion

Morphology of parents: The three parents involved in this interspecific hybrids (cultivated and two wild) have all significantly differ in morphological attributes among themselves. A commercial cultivar Sugar Baby has weak secondary leaf lobing, medium leaf blade width, sparse ovary pubescence, green ovary colour, round fruit shape and its average fruit weight (2.90 Kg) with dark green rind colour and weak fruit stripes, dark red flesh colour, black seed and having very high TSS of 9.32^o brix. Thumba being perennial in nature it has the longest crop duration more than three months i.e. 99.89 days required from pollination to harvest. Leaves of *C. colocynthis* (DWM 210) have strong secondary leaf lobing, narrow leaf blade width. It has small green colour with dense ovary pubescence. *C. colocynthis* fruits are generally small round (0.45 Kg) with yellow rind colour when ripened (Fig 1). The white flesh with numerous black seeded per fruit and highly bitter in taste (TSS 3.05^o brix). Whereas another important wild genotypes (DWM 32 & DWM 35) belongs to *C. lanatus* var. *citroides* have also differ from other two parents in morphological characters such as semi-perennial in nature and have medium leaf lobing, sparse ovary. The crop duration of these two citron melon has

87.67 & 77.03 days to reach its maturity stage. The average fruit weight of DWM 32 & DWM 35 (0.32 & 1.60 Kg) and fruits are generally diffused stripes, flat globe with slightly yellow rind colour at maturity stage. Fruits are not edible due to low in TSS at around 2.68 & 1.54^o brix respectively.

Morphology of inter specific hybrids: Female parent Sugar Baby was crossed with these 3 wild genotypes to produce their progeny. Hybrids could be established in all the combinations though per cent fruit set was low and fruit cracking was very evident. The 3 inter specific hybrids along with 4 parents were evaluated during *kharif* seasons (Aug-Nov. 2015) to characterize them for yield contributing characters and resistance to WBNV. The F₁ progeny resembles mostly with *C. colocynthis* for many characters like perennial growth habit, leaf colour and leaf lobing, flower colour, and bitter spongy pulp of fruit and bitterness of flesh as well as seeds. Some characters like fruit shape and rind pattern on ripening, shape and colour of seeds resembled to that of cultivated type Sugar Baby (Fig 1). The fruit size, seed size and flesh colour were found to be somewhat intermediate between *C. colocynthis*, *C. lanatus* var. *citroides* and *C. lanatus* var. *lanatus*.

The three different inter specific hybrids showed following intermediate characters like crop duration (84, 87.67 and 88.67), and average fruit weight (0.56 kg, 0.60 kg and 2.10 kg). The yellowish white flesh colour and low total soluble solids (TSS) i.e. (2.93, 2.40 and 3.55) bitter taste shows the dominance of these traits which are not desirable for watermelon. Only one genotype from wild progenitor DWM 210 (*C. colocynthis*) showed no disease incidence (0.0 DI) followed by inter specific hybrid (SB X DWM 210) showed DI 14.50% allowing the virus concentrations much below the detectable range at 75 DAT i.e. 0.098 and 0.120 respectively and remained almost healthy throughout all the growth stages during *kharif* season also. Other genotypes from *C. lanatus* var. *citroides* like DWM 32 and DWM 35 showed disease incidence of 23.50% and 39.50% respectively. Inter specific hybrids SB X DWM 32 and SB X DWM 35 exhibited disease incidence of 27.67% and 49.00% respectively.

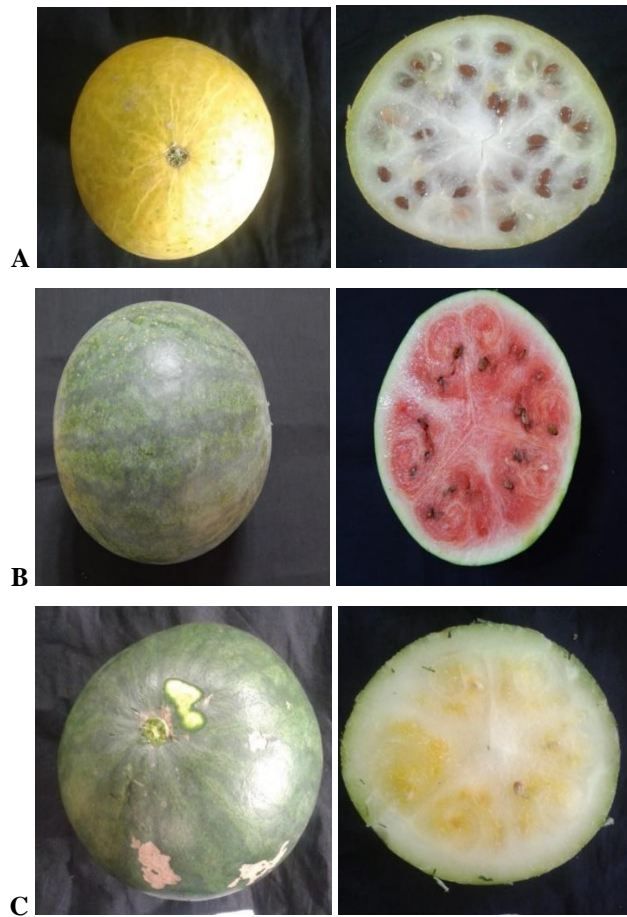


Fig 1: Morphological features of parents and their F1 progeny A) *C. colocynthis* B) Sugar Baby C) Sugar Baby x *C. colocynthis*

Table 2: Mean values for qualitative and quantitative attributes of parents and F1 hybrids of *C. colocynthis* and *C. lanatus* var. *citroides* and Sugar Baby

Characters	DWM 35 (<i>Citrullus lanatus</i> var. <i>citroides</i>)	DWM 32 (<i>Citrullus lanatus</i> var. <i>citroides</i>)	DWM 210 (<i>Citrullus colocynthis</i>)	Sugar Baby (<i>Citrullus lanatus</i> var. <i>lanatus</i>)	SB X DWM 210	SB X DWM 32	SB X DWM 35
Days to first male flower opening	17.02	21.68	44.33	17.35	15.30	15.00	13.66
Days to first female flower opening	24.05	27.61	52.05	26.02	26.66	23.00	23.00
No. of fruits per plant	3.61	10.65	9.62	4.89	8.60	6.80	6.40
Days to harvest from pollination	77.03	87.67	99.89	62.00	84.00	87.67	88.67
Average fruit weight (Kg)	1.60	0.37	0.45	2.90	0.56	0.60	2.10
Fruit shape	Flat globe	Round	Round	Round	Round	Round	Round
Fruit length (cm)	13.62	8.51	9.11	13.35	10.47	11.83	15.10
Fruit diameter (cm)	13.91	9.51	8.58	13.48	10.17	10.33	13.10
Flesh thickness (cm)	13.18	8.18	7.58	12.00	9.07	9.33	12.00
Flesh colour	Yellow	White	White	Dark red	Intermediate	Intermediate	Intermediate
Rind thickness (cm)	0.80	1.10	0.64	1.35	0.80	0.73	1.17
Seed colour	Red	Brown	Black	Black	Black	Black	Black
Yield per plant (kg)	3.9	2.34	2.58	7.99	3.05	3.01	9.06
Total soluble solids (°Brix)	1.54	2.68	3.05	9.32	2.93	2.40	3.55
Disease incidence (DI) at 75 DAT	39.50	23.50	0.00	79.05	14.50	27.67	49.00
OD at 405 nm for 75 DAT	2.210	0.630	0.098	2.102	0.120	0.230	1.890

Three inter specific hybrids between Sugar Baby (*Citrullus lanatus* var. *lanatus*) and two wild species *C. colocynthis* (DWM 210) and *Citrullus lanatus* var. *citroides* (DWM 32 & DWM 35) could be produced and evaluated for morphological traits and resistance to bud necrosis disease. These hybrids showed resemblance to wild parent for perennial growth, leaf lobbing, colour while fruit shape and

size resembled to cultivated parent. White flesh colour, low TSS and bitterness were dominant traits in inter specific hybrids which are not desirable trait for watermelon. Among three inter specific hybrids, Sugar Baby and DWM 210 (*C. colocynthis*) exhibited highly resistant reaction against bud necrosis disease as it showed 14.50 % disease incidence (DI) with the virus concentration values showed as 0.120 at 75

days after transplanting and had strong secondary leaf lobing, perennial in crop growth habit, late in flowering, longer crop duration, small fruit size, highly seeded, white in flesh colour and highly bitter in taste. Inter specific hybrid possess many undesirable traits like white flesh and bitterness. Introgression of desirable traits with minimum linkage drag or transfer of resistance from wild to cultivated genotype is a very challenging job which needs to be attempted in future breeding programmes. Therefore, Selection of genotypes with resistance to bud necrosis disease as well as possessing desirable traits of red flesh colour, sweetness and high TSS as many of these traits are governed by recessive genes can also be achieved through continuous backcross with the recipient parent. Molecular mapping of genes responsible for resistance to WBNV will be helpful in introgression of resistance gene(s) with minimum linkage drag and selection of genotype with resistance to WBNV along with desirable trait can be achieved in shorter period of time and this study is in progress.

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