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Insight into the Morphological Diversification and Viral Disease Resistance in the Interspecific Crosses of *Abelmoschus esculentus* × *Abelomsochus moschatus*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors PK, AS, MKS, DKS, AR and HS conceived the idea. Author HS performed research work, and draft the entire manuscript with author PK. Author SKT assisted in some computational work and edits the entire drafted manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Yellow Vien Mosaic Virus (YVMV) and Okra Enation Leaf Curl Virus (OELCV) are major threat of okra production in India. Due to instantly breakdown of resistance and absence of durable source of resistance in the cultivated species it's become more challenging for okra growers. Therefore, interspecific hybridization is considered as a reliable method for stable resistance. In the present experiment crossing of 7 wild accessions of *A. moschatus* and 3 cultivated okra were done during *Kharif* season of 2022-23 and crossed hybrid were grown in next year *Kharif* season. Further, field screening of 7 wild (*A. moschatus*), 3 cultivated (*A. esculentus*) and their 21 hybrids was carried out and assess morphological diversity to know the expressions of various traits at the research farms of the ICAR-IIVR, Varanasi. Out of 10 parents and 21 hybrids, only two parents and 6 hybrids were highly resistant for both YVMV and OELCV disease. The remaining 5 parents *viz*., (EC-329394, EC-360953, IC-039308, IC-469583 and IC-47737) and 15 hybrids were categorized into five distinct groups based on their disease response, ranging from Resistant (R) to Highly Susceptible (HS). The range of percent disease incidence varied from 10.00 to 90.00. Significant differences were observed for all quantitative traits for both parents and hybrids. In interspecific hybridization qualitative traits were resemble to the *A. moschatus* which indicates dominance of wild species for qualitative traits. Whereas most of the quantitative traits were express intermediate of both parent which indicate incomplete dominance for these traits. Significant differences among hybrids and parents indicate that there are great opportunities for breeders to diversify okra through interspecific hybridization.

Keywords: Okra; YVMV; OELCV; interspecific hybrids; diversity.

1. INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench) is a most edible vegetable crop belongs to Malvaceae family and possess chromosome number (2n=130). Worldwide, okra is extensively grown in tropical and sub-tropical climates [1]. Culinary and therapeutic qualities of okra play a major role in healthy diet of humans [2]. Okra is known by many different regional names all across the world. In the United States, it is referred to as gumbo, in England it is known as lady's finger and in India, bhindi. The world's largest producer of okra is India and contribute more than 60% to the global production [3]. Okra was earlier found in the Abyssinian center of origin, which encompasses the higher eastern region of the Anglo Egyptian Sudan, the highland or plateau portion of Eritrea, and modern-day Ethiopia. Immature green pods of okra are usually consumed as vegetables, but the extract from the pods can also be used to increase the viscosity of soups and sauces in many recipes [4]. *A. moschatus* is grown for its musk scented seeds as well as an ornamental plant and most polymorphic species [5]. The corolla is yellow with dark purple base in colour, and about 7 to 12 cm in diameter and the plant can be grow up to 1.5-1.6 m. Flowers are large, and typically appear solitary axillary. Fruits can be grown up to 6.5-7.5 cm long, hispid, ovate in shape, and acute. *A. moschatus* are also reported for resistance to viral diseases OELCV and YVMV [6].

Insects, fungus, nematodes, and viruses are the major biotic stresses that typically harm the okra production in India. Among them the major threat to its cultivation is the high prevalence of two viral diseases *i.e.* Okra Enation Leaf Curl Virus (OELCV) and Yellow Vein Mosaic Virus (YVMV) [7]. The losses due to YVMV may be upto 50–90% [8] and in OELCV upto 30.00-100% [9] depending upon infection stages. Nowadays, these diseases drastically decrease yields in all okra growing states of India. Developing resistant/tolerant cultivars appears to be the greatest way to reduce the loss because using insecticides and pulling off diseased plants is neither feasible nor a cost-effective way to control the virus. Due to evolution of new viral strain many viral disease resistance cultivars of okra are became susceptible [10]. Therefore, there is a need to make more attempts to produce okra cultivars that are resistant to both viral diseases. The main obstacle to creating a permanent resistant variation of okra is the absence of a reliable source of resistance to YVMV and OELCV in cultivated species. Nonetheless, it has been noted that a few okra species found in the wild can serve as consistent and trustworthy sources of resistance in which *A. moschatus* is one of them. But there were very limited efforts made to transfer resistant gene

from wild taxa to cultivated gene pool. Therefore, here is a need to make more endeavors to screen crop wild relatives (CWR) and subsequently transfer the viral disease resistance genes through interspecific crossbreeding and evaluate F_1 for both disease resistant and crop diversification. The wild relatives of okra provide not only disease resistant gene, but genetic diversity for many desirable traits that may not be available in cultivated okra. The novel genetic diversity within these wild species may be the building block for improvement of quality and productivity in okra. Continuous domestication of wild species is the vital foundation for crop improvement in any crop [11]. Hybridization plays a crucial role in generating diversity in angiosperm species [12]. Hybridization may lead to beneficial new phenotypes through rapid genomic changes [13]. As a minimum only 25% species are involved in hybridization and potential introgression with other species [14]. Keeping in view the above facts, this experiment was conducted to develop viral disease resistance and morphological diversification through interspecific crosses between *A. esculentus* and *A. moschatus*.

2. MATERIALS AND METHODS

The present experiment was laid out in Randomized Block Design (RBD) with three replications consisted of 3 blocks, each with one replication at the research farms of the ICAR-IIVR, Varanasi, which is located at 82.52°*E longitude and* 25.10°*N latitude.* The experimental materials were consisted seven accessions of *Abelmoschus moschatus viz*. EC-329394, EC-361007, EC-360953, IC-039308, IC-469583, IC-47737, EC-360095, three genotypes of cultivated okra such as Pusa Sawani, Kashi Pragati, and VRO-R-8 and their 21 hybrids. The parents (seven accessions of *Abelmoschus moschatus* and three accessions of cultivated okra) were grown during *Kharif* (Rainy) season of 2022-23 for develop 21 hybrids through crossing between seven crop wild relatives and three cultivated species. Harvested seeds of these crosses were sown during *Kharif* season of 2023-24 used for screening of viral diseases under natural epiphytotic conditions for OELCV and YVMV and morphological characterization. Observation recorded for 12 qualitative and 8 quantitative characters *viz*. general aspect, branching habit, stem pubescence, stem color, leaf colour, shape of epicalyx segments, persistence of epicalyx segments, petal color, red coloration of petal base, position of fruit on stem, fruit colour, plant height, number of branches, first flowering node, number of node, internodal length, leaf length, leaf width and fruit length at maturity. Heat map analysis was performed by using Graphpad Prism 10.

Screening for disease resistance: Observation was recorded twice at 60 days and 90 days after sowing and disease reaction were calculated on the basis of 90 DAS disease severity. The scale 0->4 is used for calculation of PDI, coefficient of infection and disease reaction as given by [15] for both diseases.

Percent disease incidence (PDI) values will be calculated by using the following formulas:

$$\boldsymbol{PDI}\ (\%) = \frac{Number\ of\ infected\ plants}{Total\ number\ of\ plants\ observed} X\ 100$$

The coefficient of infection (CI) was calculated by multiplying the PDI (YVMV and OELCV disease) and response value (RV) assigned for with severity grade value.

$$CI\ (\%) = PDI \times RV$$

Where,

PDI = Per cent disease infection.
RV = Response value.
CI (%) = Coefficient of Infection

Table 1. Scale use for screening disease in okra to OELCV and YVMV

Symptoms	Severity scale	Response value	Reaction
Symptoms absent	0	0.00	H R
Very mild symptoms up to 25% leaves	1	0.25	R
Appearance of disease between 26 and 50% leaves	2	0.50	M R
Symptom between 51 and 75% leaves	3	0.75	M S
Severe disease infection at 75% leaves	4	1.00	S
Above 75% leaves	>4	>1.00	HS

Where, HR = Highly resistant, R = Resistant, S = Susceptible, HS = Highly susceptible, MR = Moderately resistant, MS = Moderately susceptible

3. RESULTS AND DISCUSSION

The phenomenon of morphological diversification is discussed in terms of high-yielding and stable okra genotypes development for viral diseases through interspecific hybridization. Despite differences observed among interspecific crosses of different wild species, resistant hybrid for both diseases were also found by interspecific hybridization. The result obtain through present experiment is discuss below: -

Evaluation of genotypes for OELCV and YVMV resistance: Data pertaining to the evaluation of 10 parents and 21 hybrids against YVMV and OELCV and their level of resistance was given in Table 2. Based on coefficient of infection (CI %), all were grouped into 6 groups, *i.e.* Highly Susceptible (HS), Susceptible (S), Moderately Susceptible (MS), Moderately Resistant (MR), Resistant (R) and Highly Resistant (HR). Out of 10 parents and 21 hybrids screened for OELCV, two parents and seven hybrids were found as HR, four parents and four hybrids were found as R, eight hybrids were observed as MR, one parents and two hybrids were grouped as MS, two parents were susceptible and one parent was HS. The range of percent disease incidence (PDI) was varied from 10.00 % (P. Sawani × IC-47737, VRO-R-8 × IC-469583 and K. Pragati × IC-039308) to 80.00 % (VRO-R-8) at 90 DAS. Crosses which exhibited lowest (10.00 %) PDI were (P. Sawani × IC-47737, VRO-R-8 × IC-469583 and K. Pragati × IC-039308) which were found moderately resistant (MR) to OELCV Disease. Maximum percent disease incidence (80.00 %) was recorded in VRO-R-8 followed by Pusa Sawani (70.00%), Kashi Pragati (60.00%), VRO-R-8 × EC-360953 (50.00%), P. Sawani × EC-360953 (40.00%), VRO-R-8 × IC-47737 (30.00%) and K. Pragati × IC-47737 (30.00%). Six hybrids and two parents observed as no incidence of OELCV so their PDI were (0.00) hence it indicates these genotypes resist to OELCV disease due to its wild character of genotypes. These six hybrids, two parents and one other hybrids K. Pragati × IC-039308 (CI%=2.50) were categorized under (Highly Resistant) group. Maximum CI (%) in parents was recorded in VRO-R-8 (CI%=72.50) which was followed by P. Sawani (62.50%) and Kashi Pragati (60.00%) which were categorized under highly susceptible (HS). One accessions of *A. moschatus i.e.* EC-360953 (CI%=20.00) and two hybrids *viz*., VRO-R-8 × EC-360953 (CI%=32.50) and P. Sawani × EC-360953 (CI%=25.00), observed as moderately susceptible (MS). Seven hybrids *viz.,* P. Sawani × EC-329394 (CI%=15.00) followed by K. Pragati × EC-360953 (CI%=15.00), P. Sawani × IC-469583 (CI%10.00), VRO-R-8 × IC-039308 (CI%10.00), K. Pragati × EC-329394 (CI%10.00), K. Pragati × IC-469583 (CI%10.00) and K. Pragati × IC-47737 (CI%10.00) observed as moderately resistant.

Whereas, four parents *viz*., EC-329394 (CI%=7.50), IC-469583 (CI%=7.50), IC-039308 (CI%=5.00) and IC-47737 (CI%=5.00) and five hybrids *viz.,* VRO-R-8 × IC-47737 (CI%=7.50), P. Sawani **×** IC-039308 (CI%=5.00), P. Sawani × IC-47737 (CI%=5.00), VRO-R-8 × EC-329394 (CI%=5.00) and VRO-R-8 × IC-469583 (5.00%) were grouped as resistant (R). Singh et al., [16] was also reported that wild okra have disease resistant genes for pest and disease resistance. Different levels of resistance were also reported by Kumari et al., [6] against OELCV after screening of 76 accessions of *A. moschatus* in delhi conditions. Similarly, Venkataravanappa et al*.* [15] find out 125 wild accessions were highly resistant out of 178 cultivated/wild okra genotypes studies. This finding was also in agreement with [10,17].

On an account of field screening of 10 parents and 21 hybrids under field condition for YVMV, per cent disease incidence (PDI) were varied from 10% to 90 % at 90 days after sowing. Maximum percent disease was recorded for VRO-R-8 (90%) which was followed by Pusa Sawani (80%), Kashi Pragati (70%), VRO-R-8 × EC-329394 (40%), P. Sawani × EC-360953 (40%), VRO-R-8 × EC-360953 (30%), K. Pragati × IC-039308 (30%), EC-360953 and IC-469583. Plants which exhibit maximum symptoms have highest disease severity which is in accordance with findings were also reported by Venkataravanappa et al., [15]. Two parents and 6 hybrids exhibited no incidence of disease and two parents *viz*. EC-329394 and IC-47737 show less disease incidence (CI%=2.5) were grouped under highly resistance (HR) category. Three crosses *viz.,* P. Sawani × IC-469583 (CI%= 7.5), P. Sawani × IC-47737 (CI%=5.00) and VRO-R-8 × IC-47737 (CI%=5.00) were categorized under resistant (R). Whereas, three parents and ten hybrids were grouped under moderately resistant (CI%=9.10 to 19.00), and two hybrids *viz.,* P. Sawani × EC-360953 (CI%=20.00) and VRO-R-8 × EC-329394 (20.00) were grouped under moderately susceptible (MS). Among

cultivated species Kashi Pragati (CI%=60) categorized under susceptible, Pusa sawani (CI%=70) and VRO-R-8 (CI%=90.00) under highly susceptible. Similarly, Seth et al., [18] observed resistance to YVMV in wild taxa *i.e. A. caillei* and *A. manihot.* Earlier workers also reported resistance to YVMV in wild species of okra, especially in *A. tetraphyllus* [19,17,20].

Morphological characterization of qualitative traits: The morphological characterization of 10 parents and 21 hybrids of okra for 12 qualitative traits has been presented in Table 3. The morphological traits were characterized in the genotypes as per descriptors developed by IBPGR (1991) for okra.

With respect to general aspect of growth habit erect growth were recorded in all genotypes and also in their hybrids. Erect growth of plant is helpful in intensification of population and increase fruit yield. Therefore, it is desirable trait for improvement in okra. Similar finding was also reported by [21]. In terms of the branching habit Pusa Sawani, Kashi Pragati and VRO-R-8 exhibited medium type branches. Whereas, strong branches were observed in *A. moschatus* accessions *i.e.* EC-329394, EC-361007, IC-039308 and EC-360095 and medium branches were observed in EC-360953, IC-469583 and IC-47737. Out of 21 interspecific hybrids 12 hybrids exhibit strong branches whereas 9 hybrids show medium. In view of the stem pubescence glabrous pubescence was found in EC-329394, Pusa Sawani, Kashi Pragati and VRO-R-8, conspicuous pubescence was found in EC-361007, EC-360953, IC-469583, IC-47737 whereas slight pubescence was observed in IC-039308, EC-360095 in parents. In the inter-specific F_1 9 hybrids show glabrous stem pubescence whereas 12 hybrids show slight rough pubescence. As per as leaf color is concern, in out of ten parents the green with red veins leaf was recorded in EC-329394, EC-360953 and EC-360095 whereas green colour was recorded in EC-361007, IC-039308, IC-469583, IC-47737, Pusa Sawani, Kashi Pragati and VRO-R-8. Crosses made between green with red veins and green leaf, the interspecific hybrid exhibit green with red veins leaf which show the dominance of its on green color. In interspecific $F_{1,}$ green with red veins leaf was observed in 9 hybrids whereas green colour was observed in 12 hybrids. With respect to epicalyx shape, petal color, red coloration of the petal base, and position of fruit on the main stem, all parents and hybrids exhibit similar expressions, *i.e.,* linear epicalyx, yellow petals with pigmentation on both sides, and erect fruit on the stem. This revealed that the accessions of *A. moschatus* were similar to *A. esculentus* for these traits. Therefore, no significant differences were found among the hybrids for these traits. With respect to fruit color, green with red patches was observed in EC-361007, IC-039308, IC-469583, IC-47737, EC-360095 and green fruits was found in EC-329394, EC-360953, P. Sawani, Kashi Pragati and VRO-R-8 in parents. Whereas in interspecific F_1 15 hybrids exhibited green with red patches fruit and remaining 6 hybrids show green fruit. As far as fruit pubescence is concerned, slightly rough fruit pubescence was found in EC-329394, EC-360953, IC-469583 and in 6 hybrids and prickly fruit pubescence was observed in EC-361007, IC-039308 whereas downy fruit pubescence was found in IC-47737, EC-360095 and in 15 hybrids. Interspecific hybrids developed by crossing among 7 accession of *A. moschatus* and three accessions of cultivated species resembled to the *A. moschatus* for branching habit, stem pubescence, leaf color, fruit colour and fruit pubescence indicating dominance of respective expression of traits as in the pollen parent over the present in female parent. Most of qualitative traits were genetically controlled and less dependent on environmental factors. Similar results were reported by Sinha and Mishra [22] and Bashar et al. [23].

Morphological characterization of quantitative characters: The data recorded on eight quantitative traits were subjected to statistical analysis. Analysis of variance (Table 4) indicated that there were significant differences among ten parents and their twenty-one hybrids included in experiments for all the quantitative traits studies.

The perusal of data for revealed highly significant differences among all parents and hybrids for plant height which was ranging from 84.73 (cm) to 136.68 (cm) (Figs. 1 & 7) in parents and from 89.23 (cm) to 122.53 (cm) (Figs. 4 & 8) in hybrids. Wild accessions have short plant height as compared to cultivated species. In *A. moschatus* maximum plant height was observed in EC-361007 (124.67 cm) followed by EC-360953 (110.80 cm) and IC-039308 (101.49 cm). Whereas in *A. esculentus* the genotype with maximum (136.68 cm) plant height was VRO-R-8 followed by Kashi Pragati (128.35 cm), and Pusa Sawani (125.83 cm). Lowest plant height was observed for EC-

Table 2. OELCV and YVMV incidence in okra parents and their hybrids after 90 days of sowing

Genotypes	OECLV PDI @90 DOS	OELCV CI @90DOS	OELCV Reaction	YVMV PDI@90 DOS	YVMV CI @90DOS	YVMV Reaction
EC-329394	20	7.5	R	10.00	2.5	HR
EC-361007	0	0	HR	0.00	0	HR
EC-360953	20	20	MS	30.00	12.5	MR
IC-039308	20	5	R	20.00	10	MR
IC-469583	20	7.5	R	30.00	15	MR
IC-47737	20	5	R	10.00	2.5	HR
EC-360095	0	0	HR	0.00	0	HR
Pusa Sawani	70	62.5	S	0.88	70	S
VRO-R-8	80	72.5	HS	1.00	90	HS
Kashi Pragati	60	60	S	0.86	60	S
P. Sawani × EC-329394	20	15	MR	20.00	10	MR
P. Sawani × EC-361007	0	0	HR	0.00	0	HR
P. Sawani × EC-360953	40	25	MS	40.00	20	MS
P. Sawani × IC-039308	20	7.5	R	20.00	10	MR
P. Sawani × IC-469583	20	10	MR	10.00	7.5	R
P. Sawani × IC-47737	10	5	R	10.00	5	R
P. Sawani × EC-360095	0	0	HR	0.00	0	HR
VRO-R-8 × EC-329394	20	5	R	40.00	20	MS
VRO-R-8 × EC-361007	0	0	HR	0.00	0	HR
VRO-R-8 × EC-360953	50	32.5	MS	30.00	10	MR
VRO-R-8 × IC-039308	20	10	MR	20.00	10	MR
VRO-R-8 × IC-469583	10	5	R	20.00	11.66	MR
VRO-R-8 × IC-47737	30	7.5	R	10.00	5	R
VRO-R-8 × EC-360095	0	0	HR	0.00	0	HR
K. Pragati × EC-329394	20	10	MR	20.00	10	MR
K. Pragati × EC-361007	0	0	HR	0.00	0	HR
K. Pragati × EC-360953	20	15	MR	20.00	15	MR
K. Pragati × IC-039308	10	2.5	HR	30.00	10	MR
K. Pragati × IC-469583	20	10	MR	20.00	15	MR
K. Pragati × IC-47737	30	10	MR	30.00	10	MR
K. Pragati × EC-360095	0	0	HR	0.00	0	HR
Mean	21.96	13.22		14.28	13.92	
Std. Error	3.70	3.39		2.34	3.78	
S.D.	20.55	18.91		13.01	21.04	

Table 3. Morphological characterization of qualitative traits of parents and interspecific F_1

Genotype	General aspect	Branching	Stem pubescence	Stem colour	Leaf colour	Shape of epicaly× segments	Petal colour	Red coloration of petal base	Position of fruit on main stem	Fruit colour	Fruit pubescence
EC-329394	Erect	Strong	Glabrous	Green with red patches	Green with red veins	Linear	Yellow	BSR	Erect	Green	Slightly rough
EC-361007	Erect	Strong	Conspicuous	Green with red patches	Green	Linear	Yellow	BSR	Erect	Green with red patches	Prickly
EC-360953	Erect	Medium	Conspicuous	Green	Green with red veins	Linear	Yellow	BSR	Erect	Green	Slightly rough
IC-039308	Erect	Strong	Slight	Green	Green	Linear	Yellow	BSR	Erect	Green with red patches	Prickly
IC-469583	Erect	Medium	Conspicuous	Green with red patches	Green	Linear	Yellow	BSR	Erect	Green with red patches	Slightly rough
IC-47737	Erect	Medium	Conspicuous	Green	Green	Linear	Yellow	BSR	Erect	Green with red patches	Downy
EC-360095	Erect	Strong	Slight	Green with red patches	Green with red veins	Linear	Yellow	BSR	Erect	Green with red patches	Downy
Pusa Sawani	Erect	Medium	Glabrous	Green	Green	Linear	Yellow	BSR	Erect	Green	Absent
Kashi Pragati	Erect	Orthotropic	Glabrous	Green	Green	Linear	Yellow	BSR	Erect	Green	Absent
VRO-R-8	Erect	Medium	Glabrous	Green	Green	Linear	Yellow	BSR	Erect	Green	Absent
Pusa Sawani × EC-329394	Erect	Strong	Glabrous	Green with red patches	Green with red veins	Linear	Yellow	BSR	Erect	Green	Downy
Pusa Sawani × EC-361007	Erect	Strong	Slight	Green with red patches	Green	Linear	Yellow	BSR	Erect	Green with red patches	Slightly rough
Pusa Sawani × EC-360953	Erect	Medium	Slight	Green	Green with red veins	Linear	Yellow	BSR	Erect	Green	Downy

Genotype	General aspect	Branching	Stem pubescence	Stem colour	Leaf colour	Shape of epicalyx segments	Petal colour	Red coloration of petal base	Position of fruit on main stem	Fruit colour	Fruit pubescence
P. Sawani × IC-039308	Erect	Strong	Glabrous	Green	Green	Linear	Yellow	BSR	Erect	Green with red patches	Slightly rough
Pusa Sawani × IC-469583	Erect	Medium	Slight	Green with red patches	Green	Linear	Yellow	BSR	Erect	Green with red patches	Downy
Pusa Sawani × IC-47737	Erect	Medium	Slight	Green	Green	Linear	Yellow	BSR	Erect	Green with red patches	Downy
Pusa Sawani × EC-360095	Erect	Strong	Glabrous	Green with red patches	Green with red veins	Linear	Yellow	BSR	Erect	Green with red patches	Downy
Kashi Pragati × EC-329394	Erect	Medium	Glabrous	Green with red patches	Green with red veins	Linear	Yellow	BSR	Erect	Green	Downy
Kashi Pragati × EC-361007	Erect	Medium	Slight	Green with red patches	Green	Linear	Yellow	BSR	Erect	Green with red patches	Slightly rough
Kashi Pragati × EC-360953	Erect	Medium	Slight	Green	Green with red veins	Linear	Yellow	BSR	Erect	Green	Downy
Kashi Pragati × IC-039308	Erect	Medium	Glabrous	Green	Green	Linear	Yellow	BSR	Erect	Green with red patches	Slightly rough
Kashi Praagti × IC-469583	Erect	Medium	Slight	Green with red patches	Green	Linear	Yellow	BSR	Erect	Green with red patches	Downy
Kashi Pragati × IC-47737	Erect	Medium	Slight	Green	Green	Linear	Yellow	BSR	Erect	Green with red patches	Downy
Kashi Pragati × EC-360095	Erect	Medium	Glabrous	Green with red patches	Green with red veins	Linear	Yellow	BSR	Erect	Green with red patches	Downy
VRO-R-8 × EC-329394	Erect	Strong	Glabrous	Green with red patches	Green with red veins	Linear	Yellow	BSR	Erect	Green	Downy
VRO-R-8 × EC-361007	Erect	Strong	Slight	Green with red patches	Green	Linear	Yellow	BSR	Erect	Green with red patches	Slightly rough

Genotype	General aspect	Branching	Stem pubescence	Stem colour	Leaf colour	Shape of epicalyx segments	Petal colour	Red coloration of petal base	Position of fruit on main stem	Fruit colour	Fruit pubescence
VRO-R-8 × EC-360953	Erect	Medium	Slight	Green	Green with red veins	Linear	Yellow	BSR	Erect	Green	Downy
VRO-R-8 × IC-039308	Erect	Strong	Glabrous	Green	Green	Linear	Yellow	BSR	Erect	Green with red patches	Slightly rough
VRO-R-8 × IC-469583	Erect	Medium	Slight	Green with red patches	Green	Linear	Yellow	BSR	Erect	Green with red patches	Downy
VRO-R-8 × IC-47737	Erect	Medium	Slight	Green	Green	Linear	Yellow	BSR	Erect	Green with red patches	Downy
VRO-R-8 × EC-360095	Erect	Strong	Glabrous	Green with red patches	Green with red veins	Linear	Yellow	BSR	Erect	Green with red patches	Downy

360095 (84.73 cm). The hybrid which had maximum (122.53 cm) plant height was VRO-R-8 × EC-361007 followed by VRO-R-8 ×IC-47737 (118.81 cm) and VRO-R-8 × EC-360953 (117.96 cm). Whereas minimum plant height 89.23 cm was observed in P. Sawani × IC-469583, followed by Kashi Pragati × EC-360953 (92.87 cm), Kashi Pragati × EC-360095 (94.08 cm), Pusa Sawani × EC-360095 (98.14 cm) and Kashi Pragati × IC-469583 (99.63 cm). The data analysis for number of branches per plant revealed considerable differences among all parents and hybrids which were found during the present study. This traits variant ranged from 2.73 to 7.43 for ten parents (Figs. 2 & 7) and from 3.40 to 7.32 for 21 hybrids (Figs. 5 & 8). Among ten parents the maximum number of branches 13.02 was observed in IC-039308 followed by EC-361007 (12.01 cm), EC-329394 (9.10 cm), EC-360953 (8.72 cm) and EC-360095 (8.15 cm). While minimum number of branches 2.73 was observed in VRO-R-8 followed by Kashi Pragati (3.53) and Pusa Sawani (3.92). In interspecific hybrids maximum number of branches 7.32 was observed in Pusa Sawani × EC-361007 which was followed by Kashi Pragati × IC-039308 (6.65) and VRO-R-8 × EC-361007 (6.58). While minimum number of branches 3.40 was observed in Kashi Pragati × EC-360095.

With respect to first flowering node perusal of data revealed noticeable variation in parents (Figs. 2 & 7) and hybrids (Figs. 5 & 8). It ranged from 3.67 to 16.40 with mean value 9.48 in parents and from 16.78 to 28.22 in 21 hybrids. The maximum first flowering node 16.40 was recorded in EC-329394 followed by EC-361007 (16.06), IC-47737 (13.15), IC-469583 (11.53), EC-360953 (11.22). While minimum first flowering node 3.67 was observed in VRO-R-8 followed by Pusa Sawani (4.37) and Kashi Pragati (4.43). In interspecific hybrids maximum first flowering node 7.68 was recorded in Kashi Pragati × IC-039308 which was followed by Pusa Sawani × EC-329394 (7.64) and Pusa Sawani × IC-47737 (7.36). While minimum first flowering node 3.93 was observed in P. Sawani × IC-039308. Like first flowering node, significant differences were present in respect to the number of nodes. It ranged from 19.43 to 32.19 (Figs. 2 & 7) in parents and from 16.78 to 28.22 in hybrids (5 & 8). The genotypes with maximum number of nodes 32.19 was recorded in VRO-R-8 followed by IC-039308 (29.56), Kashi Pragati (29.16) and Pusa Sawani (26.94). The minimum number of nodes 19.43 was recorded in IC-47737 followed by EC-360095 (21.68) and IC-469583 (22.65). The genotypes that maximum number of nodes (28.22) was recorded in VRO-R-8 × IC-039308, followed by VRO-R-8 × EC-329394 (26.31) and Kashi Pragati × IC-039308 (25.55). While minimum number of nodes (16.78) was recorded in Kashi Pragati × EC-360953, followed by Pusa Sawani × EC-361007 (16.82) and Kashi Pragati × EC-360095 (17.54).

In terms of internodal length, the parents under study showed considerable differences as well as for their hybrids. The length of internodes varied significantly, ranging from 3.05 cm to 5.10 cm (Figs. 3 & 7) in parents and from 3.49 cm to 5.33 cm in hybrids (Figs. 6 & 8). The genotype IC-039308 (5.10 cm) had maximum internodal length, followed by Kashi Pragati (4.49 cm), Pusa Sawani (4.47 cm), VRO-R-8 (4.46 cm) and IC-47737 (4.37 cm). The minimum internodal length was recorded in EC-329394 (3.05 cm), EC-360095 (3.36 cm), EC-361007 (3.55 cm), IC-469583 (3.73 cm) and EC-360953 (4.00 cm). Whereas the interspecific hybrid, Kashi Pragati × IC-47737 had maximum internodal length 5.33 cm followed by VRO-R-8 × IC-039308 (5.13 cm) and VRO-R-8 × EC-360953 (5.01 cm). The minimum internodal length was recorded in the hybrid Pusa Sawani × EC-361007 (3.49 cm) followed by VRO-R-8 × EC-329394 (3.52 cm), Pusa Sawani × EC-360953 (3.57 cm). Analysis of data indicated significant differences in leaf length and width. The leaf length was ranging from 6.86 cm to 13.81 cm (Figs. 3 & 7) in parents, and from 6.76 cm to 15.52 cm in interspecific crosses (Figs. 6 & 8). The highest leaf length was recorded in EC-329394 (13.81 cm), followed by Pusa Sawani (11.29 cm), IC-469583 (10.23 cm), VRO-R-8 (9.63 cm) and IC-47737 (9.47 cm). The minimum leaf length was recorded in EC-360953 (6.86 cm), EC-36100 (7.00 cm), Kashi Pragati **(**8.21 cm). Whereas for the crosses, the highest leaf length was recorded in VRO-R-8 × EC-329394 (15.52 cm) followed by Kashi Pragati × EC-329394 (14.34 cm) and Kashi Pragati × IC-47737(14.26 cm). The minimum leaf length was recorded in the hybrid P. Sawani × IC-039308 (6.76 cm). Leaf width was ranging from 11.16 cm to 17. 31 cm (Figs. 3 & 7) in parents and from 10.23 cm to 17. 76 cm in hybrids (Figs. 6 & 8). The genotypes EC-329394 had maximum (17.31 cm) leaf width followed by IC-469583 (15.71 cm), Pusa Sawani (14.17 cm), VRO-R-8 (14.02 cm) and IC-47737 (13.23 cm) while EC-361007 (11.16 cm) followed by Kashi Pragati (12.23 cm) had minimum leaf

width (cm). The hybrids Kashi Pragati × IC-47737 had maximum (17.76 cm) leaf width followed by VRO-R-8 × EC-329394 (17.30 cm), Kashi Pragati × EC-329394 (16.67 cm), while P. Sawani × IC-039308 (10.23 cm) had minimum leaf width (cm). The data on fruit length at maturity (cm) revealed highly significant differences across all the parents studied (Figs. 3 & 7). The fruit length at maturity varied from 5.53 cm and (12.07 cm) in parents and from 6.76 cm and 8.84 cm in hybrids. The maximum fruit length at maturity 12.07 cm was recorded in P. Sawani followed by Kashi Pragati (11.66 cm), VRO-R-8 (9.33 cm), EC-360953 (8.38 cm) and IC-47737 (7.85 cm) the minimum fruit length at maturity was obtained from EC-329394 (5.53 cm) and IC-039308 (5.61 cm). The maximum fruit length at maturity (8.84 cm) was recorded in Kashi Pragati × IC-47737 the minimum fruit length at maturity was obtained from Pusa Sawani × EC-361007 (6.76 cm) followed by Kashi Pragati × EC-361007 (6.79 cm). For morphological traits the interspecific hybrid exhibited intermediate expression as compared to both of its parents, namely *A. esculentus* × *A. moschatus* for plant height, number of branches, first flowering node, number of nodes, internodal length and fruit length at maturity. Similar results for fruit length were reported by [21] in okra and [24,25] in brinjal. On the contrary leaf length and leaf width were more in F_1 hybrid than the parents. Similarly, result found for leaf length and width were also reported by [26]. These results offer important insights into the genetic composition of the hybrids and their potential for breeding new cultivars with advantageous traits. Successful hybridization between cultivated and wild species within the same genus relies on perfect coordination between the gene complexes of pollen and ovule parents, as emphasized by Kuboyama et al. [27]. Notably, natural amphidiploids of *A. esculentus* and *A. manihot* have been reported by Siemonsma [28], while artificial amphidiploids have also been developed using wild species [29]. However, compatibility varies among species, with *A. moschatus* [5] and *A. manihot subsp. tetraphyllus var. pungens* [30] showing high incompatibility with *A. esculentus*. In contrast, *A. tetraphyllus*, *A. tuberculatus*, and *A. caillei* have produced viable hybrids with varying degrees of fertility [31,32]. Pathak and Bal [33] also conducted a comprehensive characterization of the interspecific F_1 hybrid between *A. esculentus* and *A. manihot*, examining various morphological and agronomic traits. Similarly, Ogwu et al. [34] demonstrated the effectiveness of morphological characterization in distinguishing between *A. caillei* and *A. esculentus*, highlighting the significance of using morphological characteristics to accurately classify plant genetic resources [35,36].

Heat map analysis: In this experiment, a heatmap was made on the basis of performance of 10 parents and 21 hybrids to determine the overall performance of hybrids for 8 quantitative characters (Figs. 7 & 8). The rainbow colour conveyed the magnitude of mean performance to identify the area of high or low values. Each cell within the grid is filled with rainbow colour that corresponds to the value of the mean it represents Violet, Indigo, Blue, Green, Yellow, Orange and Red from down to up. The violet color showed the higher values while the red colour showed the lower values of mean for different traits.

Table 4. Analysis of variance for different characters of okra

S.No.	Genotypes	Mean Sum Square		
		Replication	Treatment	Error
	D.F.	2	30	60
1	Plant Height	78.253	402.233**	99.866
2	Number of branches	0.110	16.415**	1.325
3	First flowering node	0.603	35.679**	1.427
4	Number of node	0.618	33.317**	5.171
5	Internodal length	0.029	1.039**	0.183
6	Leaf length	2.113	16.102**	1.411
7	Leaf width	0.758	10.82**	1.128
8	Fruit length at maturity	1.003	5.974**	0.712

*** Significant at 5% level*

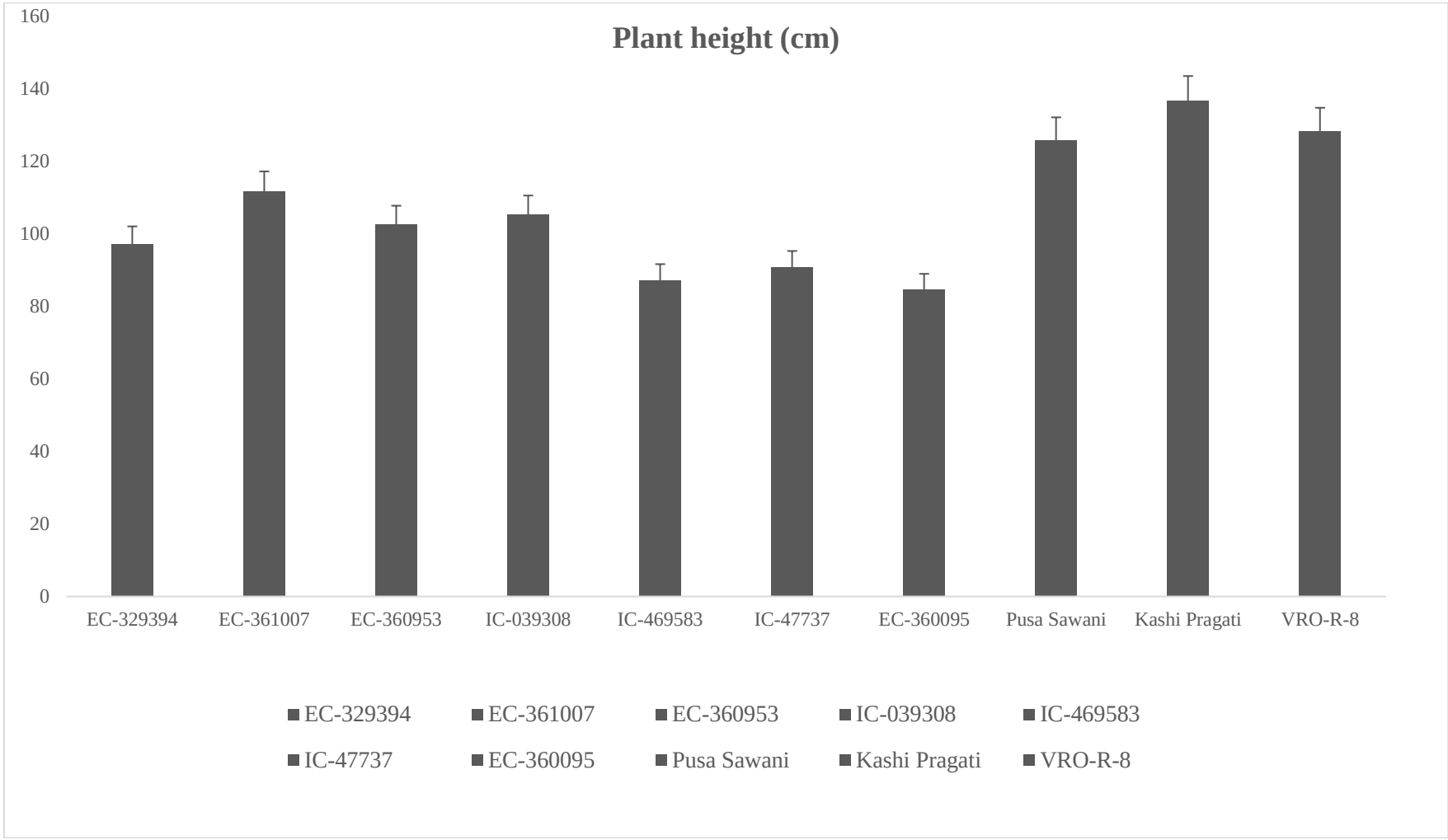

Fig. 1. Plant height of ten parents

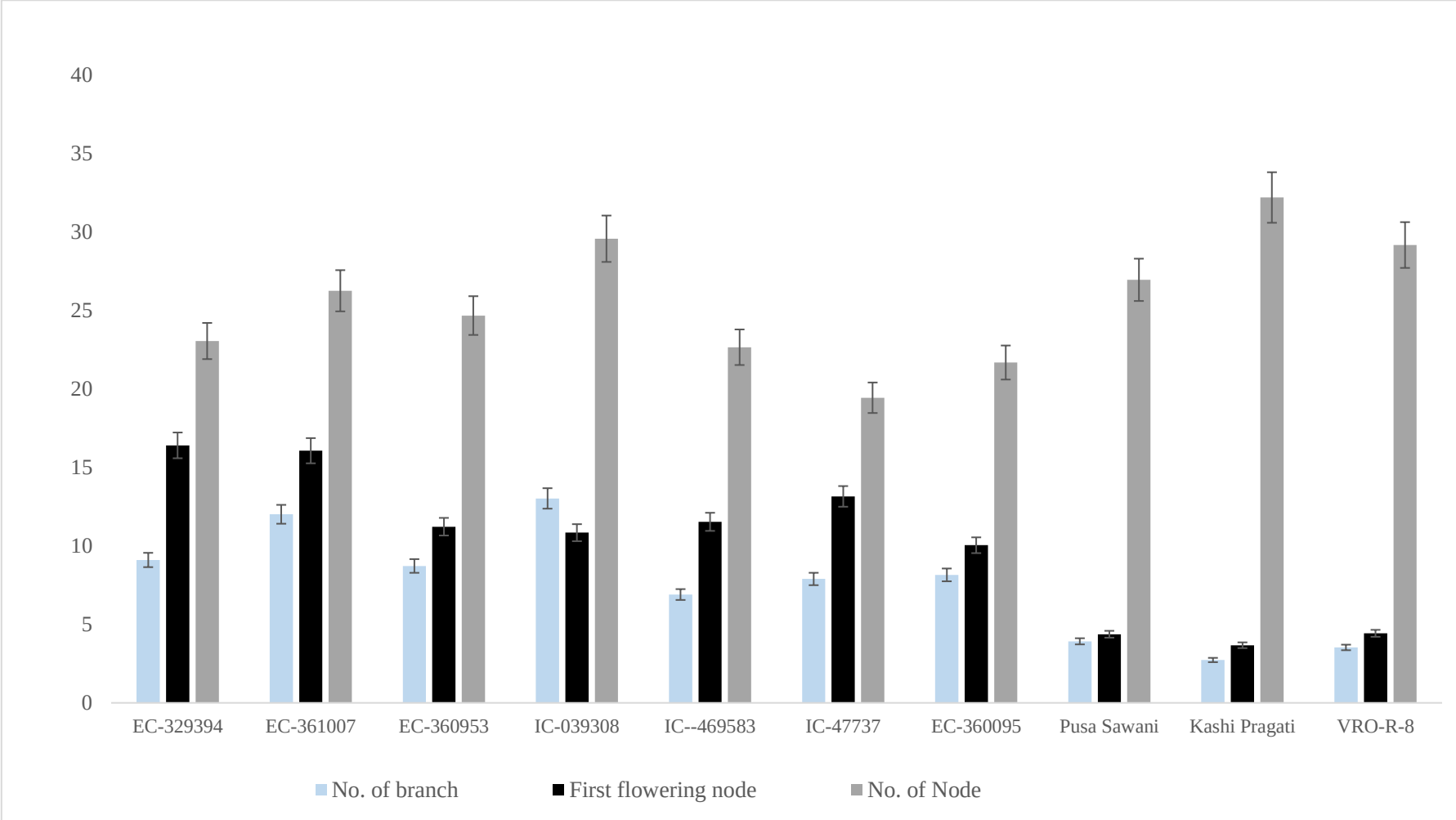

Fig. 2. Number of branches per plant, first flowering nodes and number of nodes per plant of ten parents

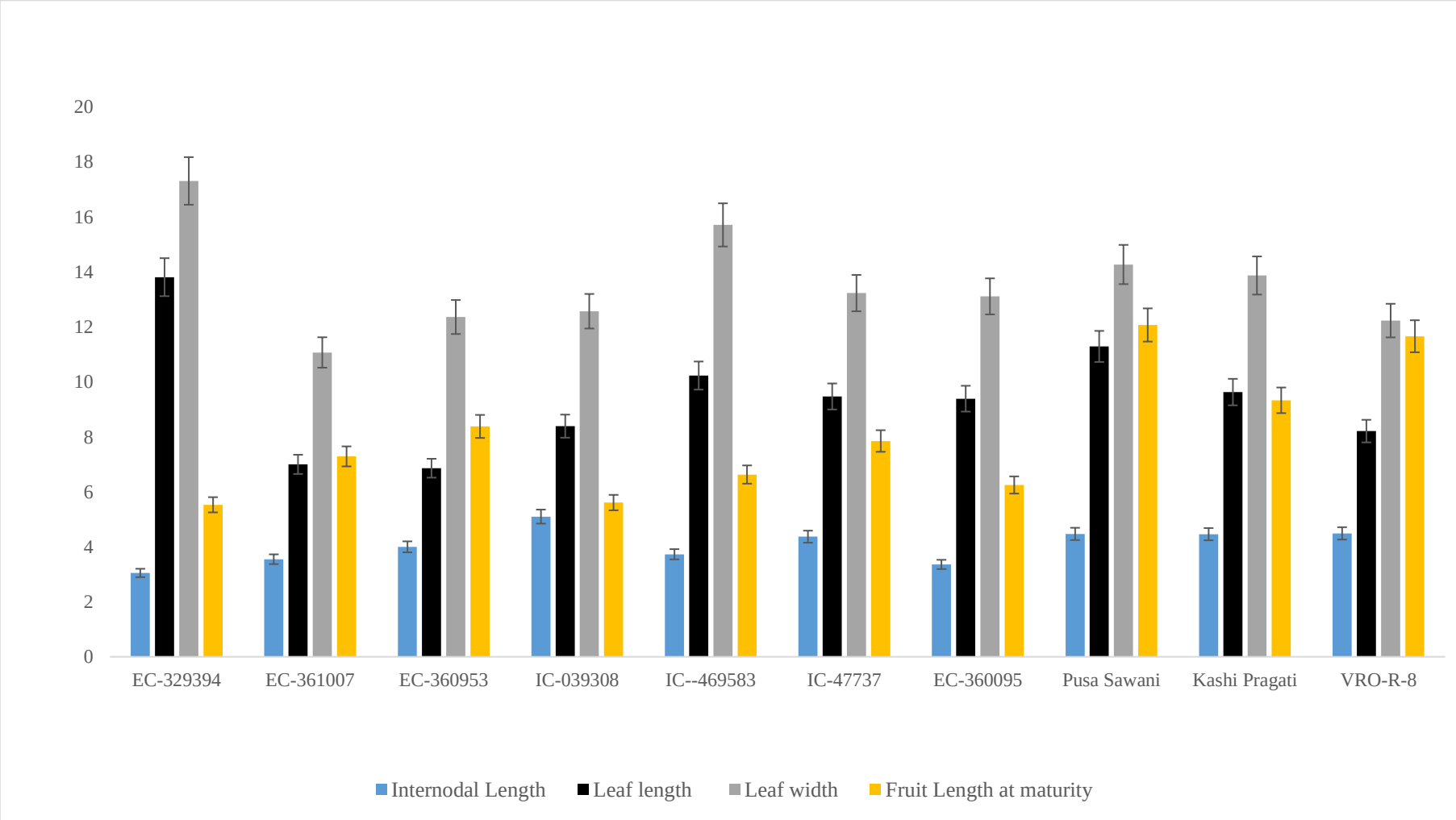

Fig. 3. Internodal length, leaf length, leaf width and fruit length at maturity of ten parents

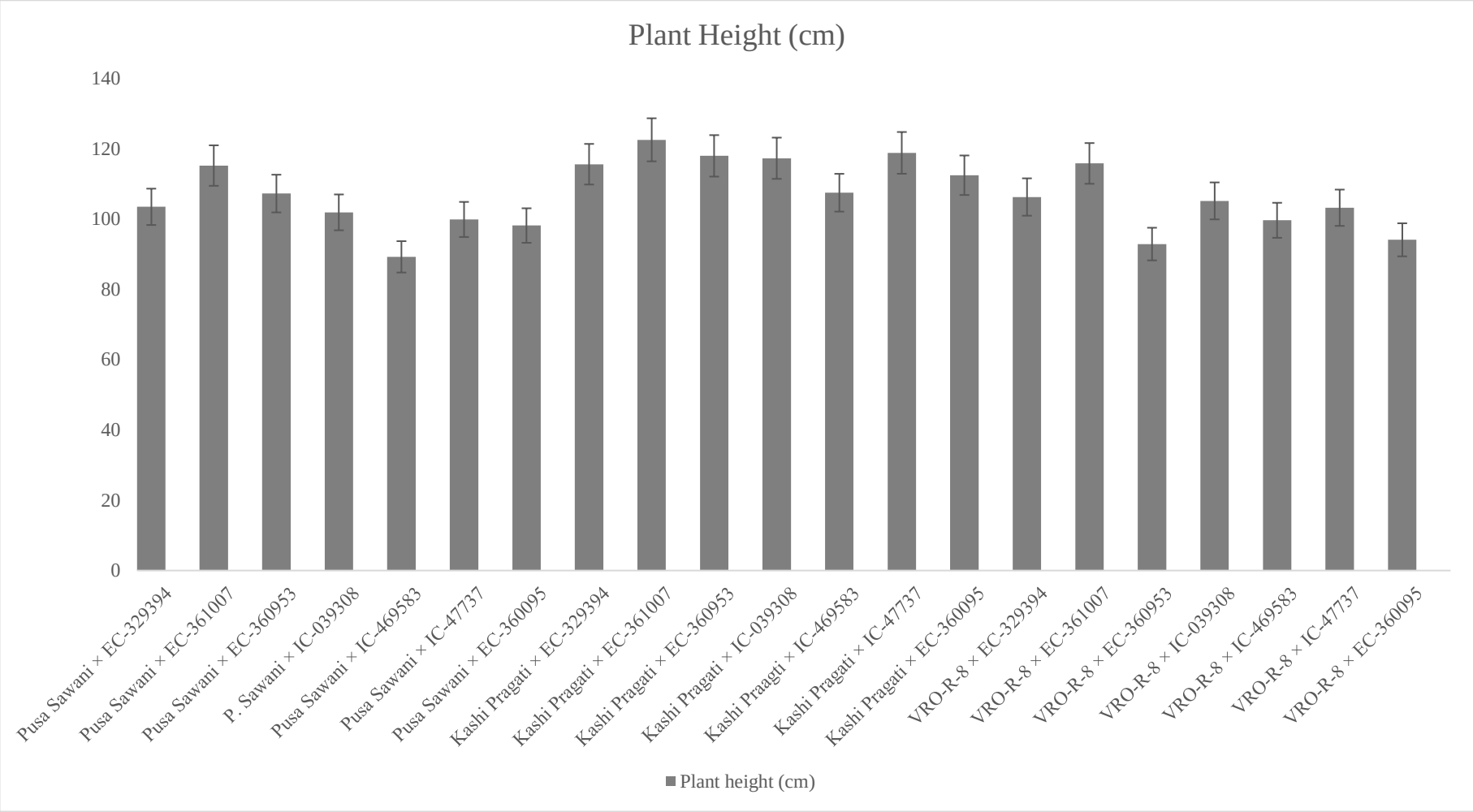

Fig. 4. Plant height of 21 interspecific hybrids

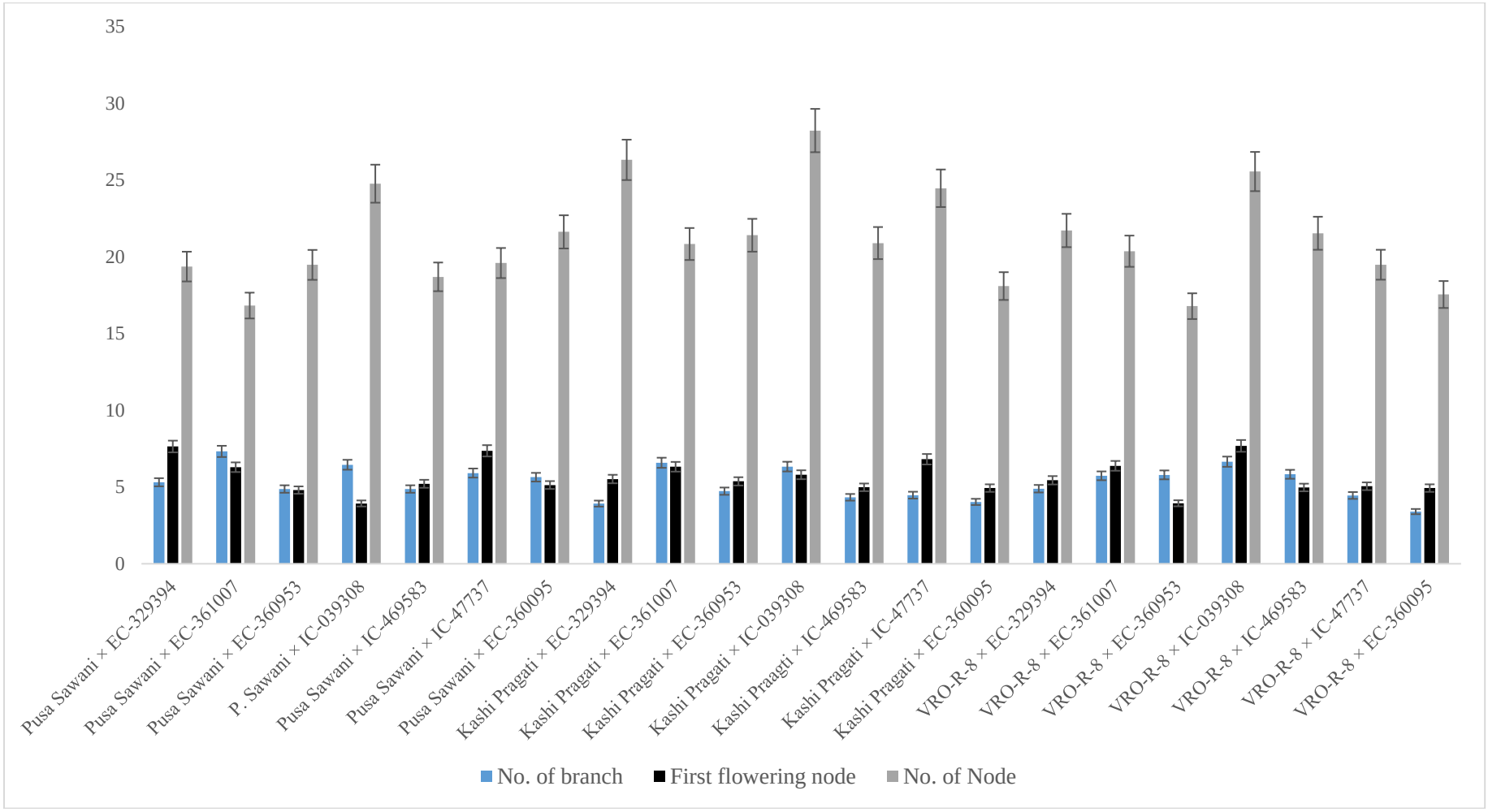

Fig. 5. Number of branches, first flowering node and number of node of 21 hybrids

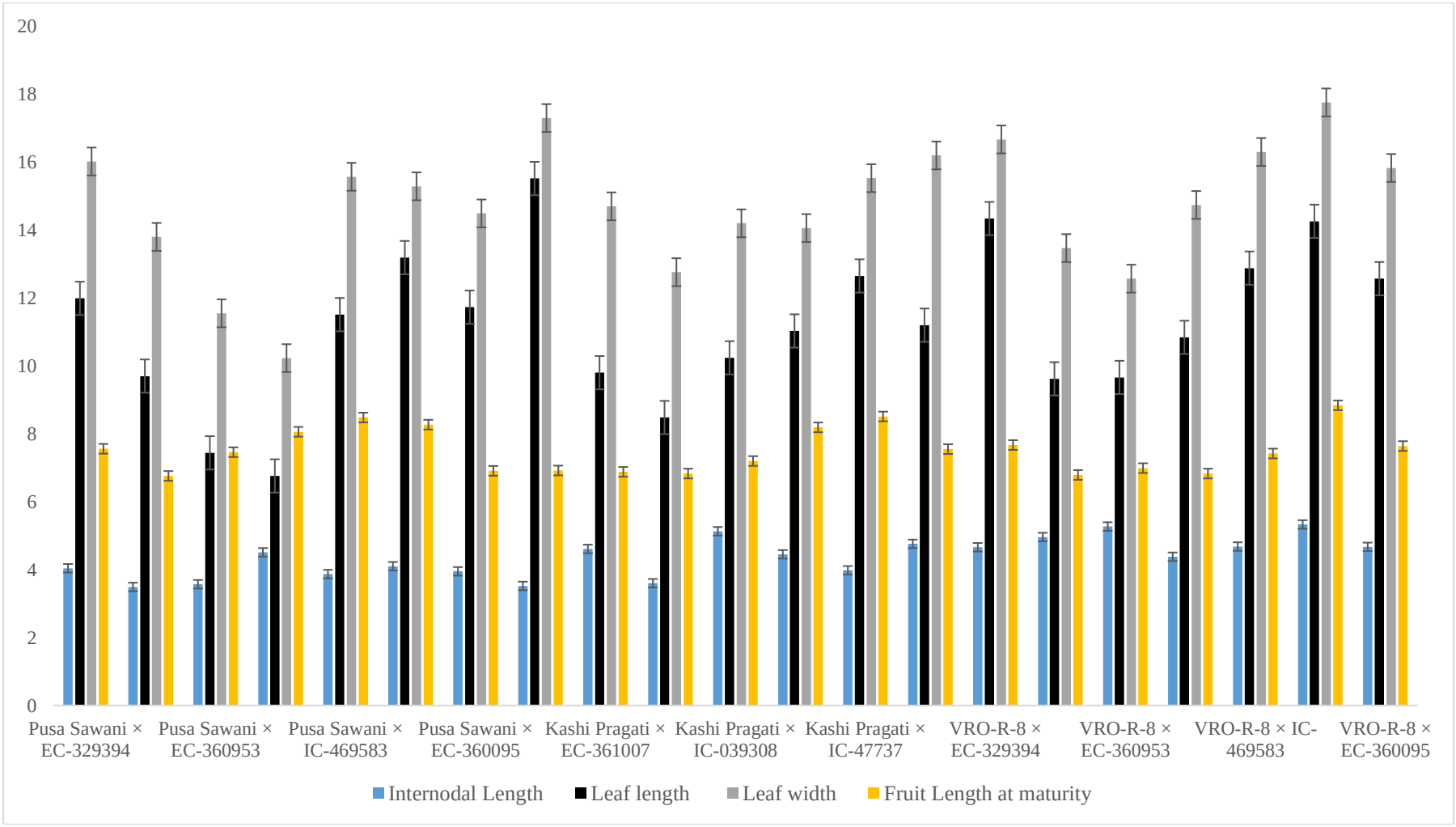

Fig. 6. Internodal length, leaf length, leaf width and fruit length at maturity of 21 hybrids

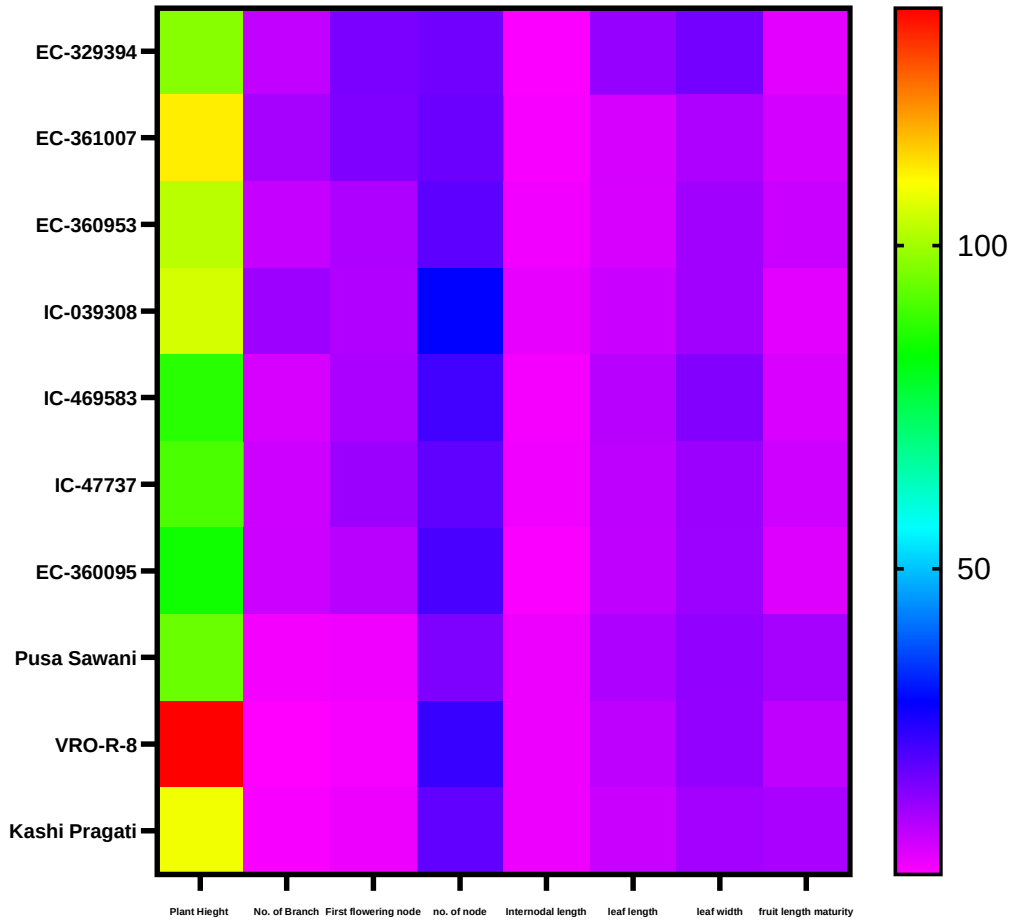

Fig. 7. The Heat map represent the divergence level among the ten parents for respective quantitative traits. The "x" axis is showing the 8 traits and "y" axis is showing the parents. Different color intensity represents the divergence level

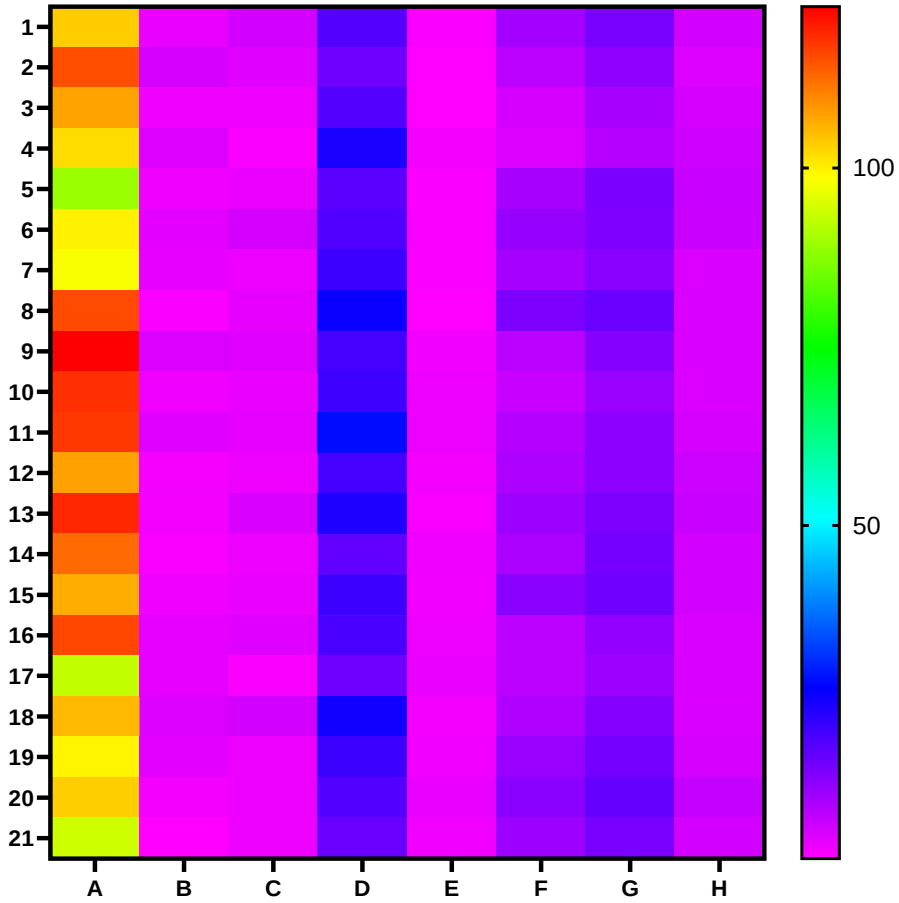

Fig. 8. The Heat map represents the divergence level among the ten parents for respective quantitative traits. The "x" axis is showing the 8 traits and "y" axis is showing the serial number of crosses. Different color intensity represents the divergence level

(In Y-axis Alphabets denotes traits viz., A-Plant Height, B-Number of Branch, C-First Flowering Node, D-Number of Node, E- Internodal Length, F-Leaf Length, G-Leaf Width, H-Fruit length at maturity)

4. CONCLUSIONS

On the basis of present investigation, it may be concluded that two accessions of *A. moschatus* were free from YVMV or OELCV symptoms under field epiphytotic conditions, and they are thought to be highly resistant. A complex screening strategy that includes hybrid derivatives in hotspots in order to identify suitable donors. Out of 21 hybrids 6 hybrids were highly resistant for both diseases. The genotypes with dual resistance (YVMV and OELCV) would be more useful. These wild genotypes can be utilized as one of the parents in interspecific hybridization program to develop a resistant variety or in the development of pre-breeding material. Morphological characterization of 10 parents and 21 hybrids shows significant differences among them and provides a useful tool for plant breeders to achieve crop diversification through interspecific hybridization.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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