Review Article

Recent Advances in Crop Improvement of Chilli (*Capsicum annuum*L.) for High Fruit Yield and Quality

ABSTRACT

_Capsicum annuum_L. is an indispensable spice cum vegetable crop cultivated worldwide for its edible fruit. The current research is majorly focused on the development of improved chilli varieties to achieve high fruit yield and quality to meet the rapidly growing market demands. Therefore, the screening of the chilli landraces and local cultivars for the genetic variation would provide best parents for the chilli crop improvement. Most of the recent chilli breeding programmes involve the assessment of genetic variation among the genotypes by the estimation of important genetic variation parameters such as phenotypic and genotypic coefficients of variation, heritability and genetic advance, gene flow, and cluster analysis. Correlation and path coefficient analysis was also applied to evaluate the progeny to understand the association between desired traits. The popular DNA-based molecular marker system, simple sequence repeat, is predominantly applied to understand the genetic variation as well as to select the desired progeny. ~~Here~~In this current review, we discussed such recent research reports on chilli crop improvement for high fruit yield and quality, highlighted the recent major achievements and provided the important information ~~useful~~ for the chilli breeders. We hope this current review would shed light on recent developments in chilli crop improvement and help chilli breeders for their future breeding programmes.

Key words: *Capsicum annuum*L., Genetic diversity, Capsaicinoids, Germplasm, SSR Markers

1. INTRODUCTION

Chilli (*Capsicum annuum* L., $2n = 2x = 24$) is an important spice cum vegetable crop with a great commercial value~~,~~ andbelonging to the family Solanaceae. Chilli fruits are regularly used both as fresh and dried in the culinary for the recipes of numerous food preparations due to their pungency and flavor. Chilli cultivation takes place around the globe both in tropical and subtropical regions and can be grown during both summer and winter seasons. In 2020, the worldwide green chilli production reached 36 million tons, with China being the largest producer (46% of the total). However, India remains as the largest producer of dried chilli in the world with 1.7 million tons recorded in 2020 [1]. In India, Andhra Pradesh is the largest producer of chilli followed by the other states, Telangana, Madhya Pradesh, Karnataka, and Odisha [2]. Indian Chilli varieties that are majorly cultivated include, BhutJolokia, Kashmiri, Guntur chilli, Jwala, Kantari, Byadagi, Ramnad, Dhani, Tomotochilli, Madras Puri, Khola and DalleKhursani. BhutJolokia (Ghost Pepper) is one of the hottest chilli in the world that is majorly cultivated in India [3].

Chilli~~crop~~ varieties differ majorly in pungency (sweet, moderate and hot), size, shape, colour and yield of the fruit, thus this polymorphism offers a great scope for developing new chilli cultivars with high fruit yields as well as with the desirable traits. The chemical compound ‘Capsaicin’ present in the chilli fruits is responsible for the pungency whereas Oleoresin is responsible for the colour and flavour of the chilli fruits. In addition, vitamin C is also present in chilli fruits. These compounds vary in quantity among the genotypes, thus, prior knowledge on phenotypic and genotypic variance is crucial for the selection of best parents for the chilli breeding programmes that aim to improve the fruit quality. Nonetheless, another major focus of the chilli breeders is to achieve the high fruit yield which is a complex hereditary character being influenced by various other traits [4]. The genetically different genotypes have a great significance to be utilized for the breeding programmes [5]. Therefore, the success of a chilli breeding is greatly dependent on the extent of genetic variation expressed by the yield contributing traits in the germplasm [6].

The estimation of genetic diversity parameters such as phenotypic and genotypic coefficients of variation, heritability and genetic advance, gene flow, and cluster analysis is crucial to understand the existing genetic variation among the genotypes [7]. Moreover, correlation and path coefficient analysis provide valuable information on interactions among the various traits at the genetic level [8]. Among the molecular markers used for evaluation of genetic diversity at the molecular level, the ~~PCR~~ PCR-based DNA marker named simple sequence repeat (SSR) is widely used in chilli breeding for the characterization of hybrids at the molecular level. SSR markers are highly polymorphic, highly reproducible, co-dominant, multi-allelic, and distributed throughout the genome, thus the SSR markers are very useful in selection of chilli hybrids with desired characteristics [9-10].

In the current review, we highlighted the latest research achievements in the crop improvement of *Capsicum annuum* L. for high fruit yield and quality. The pre-requisite of the knowledge on the existing genetic variation among the chilli genotypes is discussed. The advantages of estimation of genetic variation parameters, heterosis and correlation and molecular marker analysis in the chilli breeding for the selection of the best performing hybrids are discussed. In addition, this review presents the future perspectives of chilli crop improvement.

2. FRUIT YIELD OF CHILLI

In order to evaluate the performance of new hybrids in chilli breeding, a breeder should record various yield and yield contributing parameters such as Germination percentage (GP), First bifurcation length (FBL) [cm], Number of primary branches (NPB), Number of secondary branches (NSB), Plant height (PH) [cm], Stem diameter (SDM) [mm], Number of leaves per plant (NLP), Days to first flowering (DF), Days to first fruit maturity (DFM), Number of fruits per plant (NFP), Fruit length (FL) [mm], Fruit width (FW) [mm], Pedicle length (PL) [mm], Single fruit fresh weight (FW) [g], Single fruit dry weight (FDW) [g], Number of seeds per fruit (NSF), Hundred seeds weight (HSW) [g], Fruit wall thickness (FWT) [mm], Yield per plant (YLD) Relative chlorophyll content (RCC), Photosynthesis rate (PR), Stomatal conductance (SC), Transpiration rate (TR), etc. [11].

The selection of best parents is very important in order to develop advanced hybrids in a breeding programme. At the Institute of Tropical Agriculture, Malaysia, in order to select the best performing parents, 27 *C. annuum* mutant lines derived from two varieties (ChilliBangi 3 and ChilliBangi 5) were evaluated for 23 morpho-physiological and yield traits in their M_4 generation planned in a randomized complete block design (RCBD). A higher Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV), combined with moderate to high heritability and genetic advance were observed in various yield traits for mutant lines. Among the evaluated genotypes, genotype 2 recorded the highest fruit yield per plant whereas genotype 17 recorded the highest green fruit weight (Table 1). Overall, these findings recommended that prior evaluation of mutant parental lines is very helpful for breeders to select the best performing parents for successful execution of their future breeding programmes [11].

In Egypt, at the Experimental Farm (Kaha), Horticulture Research Institute, six diverse chilli lines (P1 to P6) have been produced by selfing, which are then crossed in half diallel mating to produce 15 F_1 hybrids under unheated greenhouse conditions. Estimated heterosis data showed the high and positive mid parent heterosis for average fruit weight whereas high and negative heterobeltiosis for fruit length and total yield. The P3 chilli line was found to be a good general combiner based on general combining ability (GCA) values and the cross P1 x P6 achieved high specific combining ability (SCA) for all the tested economic traits [12].

At the University of Peshawar, Pakistan, thirteen locally developed chilli hybrids have been evaluated along with two advance lines and seven international hybrids (P6, Atlas#3, Atlas Imp, Bonanza, Appolo, KHHP-81 and KHHP-82) in the field experiments (RCBD). The highest fresh fruit yield was recorded for local chilli hybrid CH-15140 (141 t/ha) followed by two international hybrids, Bonanza (119 t/ha) and Atlas (102 t/ha). These early maturing genotypes produced highest fresh fruit yield while seeds per fruit and pericarp thickness positively contributed towards fresh fruit yield as evident from their significant values of correlation coefficients both at the genotypic and phenotypic levels. The observed high heritability ($\geq 95\%$) along with high genetic advance ($\geq 40\%$) indicated the actuality of considerable variability among the genotypes [13]. A recent similar study that focused on evaluation of local chilli populations conducted at Urmia University, Iran cultivated 30 local chilli populations in pot conditions (RCBD design) in a research greenhouse and estimated the correlation and variance components (heritability, GCV, PCV, ECV) as well as cluster analysis. The results showed PCV and GCV were highest for single fruit weight. Pulp weight and fruit yield showed highest significant positive correlation coefficient whereas single fruit weight and fruit number exhibited highest significant negative correlation. Overall, based on the entire data the local populations such as Bash Ghala, Nakhchivan, and keshtiban were found to be superior for the most of the desired traits tested, thus recommending these lines for consideration as the top parents in further chilli breeding experiments [14].

Recently, in a greenhouse at the Federal University of Viçosa, Brazil, heterosis and reciprocal effects for agronomic and fruit traits in chilli have been evaluated by conducting the breeding experiments using two varieties of *C. annuum* (sweet bell pepper 'Cascadura Ikeda' and pungent pepper 'Jalapeno') and two varieties of *C. chinense* (sweet cultivar 'Biquinho' and pungent cultivar 'Habanero'). Both intra and interspecific F_1 hybrids (reciprocal crosses) were generated in a full diallel crossing scheme and their data on 31 phenotypic traits and 32 size and shape parameters were recorded. Data analysis revealed the significant heterosis and reciprocal effects for some of the hybrids and strong influence of seed size on initial growth vigour. Nonetheless, fruit size and shape traits were impacted by hybridization, majorly due to infertility which was restored by pollination of hybrids with parental pollen. Overall, these findings shed light on the importance of adequate combination of parental genotypes and the optimal crossing direction on achieving heterosis, yield and fruit morphology [15]. Another study by the same group of researchers using same intra and interspecific chilli hybrids evaluated the heterosis for capsacinoids contents and found that parent-of-origin effect plays a key role in determining the capsacinoids accumulation in the hybrids [16]. At the Cukurova University, Turkey, similar interspecific hybridization studies have been conducted by crossing between *C. annuum*(253A and İnan3363 varieties)and *C. chinense* (PI 159236 variety). 54 morphological traits were evaluated and analyzed for genetic variation. The results showed that the generated interspecific hybrids exhibited superior heterosis and high genetic diversity due to the gene flow to the shrinking *C. annum* genetic base, thus strongly recommending the necessity of the interspecific hybridization in chilli crop improvement [17].

At the University of Horticultural Sciences, Bagalkot, Karnataka, India, 64 ByadgiDabbichilli genotypes have been evaluated for their performance in a RCBD layout and estimated the phenotypic and genotypic correlation. Data analysis revealed the significant and positive correlation of the traits such as plant height, number of primary branches and secondary branches, fruit weight, number of fruits per plant with the fruit yield per plant and hence recommended the direct selection of hybrids based on these traits would be beneficial for the chilli crop improvement [18]. The evaluation of genetic variation of indigenous chilligermplasm is very important in order to utilize in future breeding programmes. Therefore, a recent study at Anand Agricultural University, Gujarat, India has been focused on assessment of 30 indigenous chilli genotypes to examine their genetic diversity. The genotype, ACS-08-09 recorded highest dry fruit yield among all others, however based on the genetic variability parameters the genotypes such as ACS-18-02, ACS-18-08, AnandTej, GP-93 and GAVC 112 were also found to be promising to consider to future breeding programmes (Fig. 1B) [19].

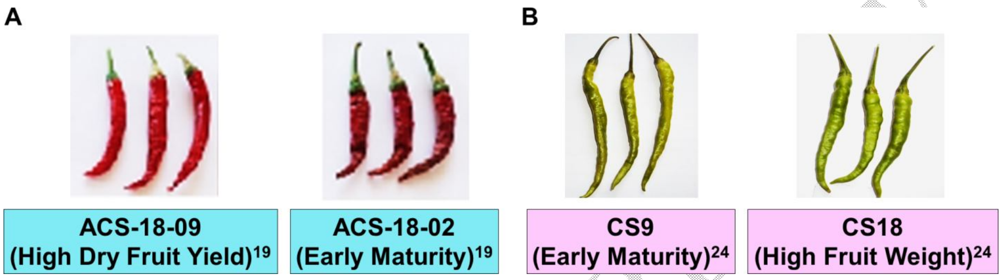

Figure 1. Recently evaluated genotypes of chilli (*Capsicum annuum*L.). (A) Promising indigenous chilli genotypes of Gujarat. (B) Promising chilli landraces of North-Western Himalayas. (Photos were adapted from their respective references cited in the figure).

Comment [F1]: The photos were not clear enough, especially A.

The evaluation of yield and yield contributing traits for 256 F_3chilli genotypes (along with 4 check varieties) has been carried out at Odisha University of Agriculture and Technology, Odisha, India. Mean performance data analysis showed that most of the genotypes were found to be better performed than the best check (BHP-143) variety. The number of fruits per plant was found to be in the range of 138 to 663, whereas Yield per plant (g/plant) was found to be within the range of 321 to 1798 for the tested genotypes. Another important yield trait, Dry yield per plant (g) was achieved in the range of 92 to 343. Various genotypes exceeded the performance of best check for certain traits. Among all the genotypes, Plant number 91 was found to be the best genotype for all traits except fruit width and pedicel length. Overall, the results of this study would help design the future chilli breeding experiments aimed to achieve high fruit yields [20]. Another recent similar study on evaluation of chilli yield tested 35 genotypes and estimated the genetic divergence using Mahalanobis's D^2 statistics. The genotype IC-347044 recorded the maximum fruit yield per plant (0.82 kg). Nevertheless, among the grouped six clusters, the genotypes belonging to the cluster IV (IC-526448, EC-378632, IC-561622) and V (IC-394819, IC-570408, EC-378688, IC-528442) were found to have most of the desired traits and could be utilized for future heterosis breeding to develop superior high yielding chilli hybrids [21].

3. FRUIT QUALITY OF CHILLI

Capsaicinoids are responsible for the pungent nature of chilli fruits and they include capsaicin and dihydrocapsaicin, a saturated hydrocarbon type of capsaicin. The content of capsaicinoids is regulated by various genes such as Ketoacyl-ACP reductase (KR), putative Aminotransferase (p-AMT), Pungency 1 (Pun1), and MYB domain transcription factors as well as several unknown genes. The parameters such as nutrient availability, light conditions, plant stress, and various environmental conditions also affect the capsaicinoid content of the chilli fruit. Therefore, chilli breeders have not been successful in developing chilli cultivars with a specific level of capsaicinoids in order to control the fruit pungency in different cultivars. However, the recent advancements in Genomic selection (GS) models developed by training with genotypic and phenotypic data of several *Capsicum* accessions has been shown to be promising for accurate prediction of capsaicinoid content of a genotype using genome-wide single nucleotide polymorphism (SNP) markers [22].

In order to evaluate the chilli genotypes for sustaining the conditions at North Western Himalayan conditions of India, a chilli breeding progamme has been conducted using six inbreds (with resistance to bacterial wilt) and their 30 cross-combinations in Hayman’s diallel analysis and estimated the genetic variation and the degree of dominance. Data analysis revealed that parents possess a predominance of dominant alleles for 50 percent blossoming, days to first harvest, harvest duration, average fruit weight, fruit yield and bacterial wilt incidence, thus indicating degree of dominance was more than one for majority of traits [23]. Another recent study conducted at North Western Himalayas on the evaluation of genetic diversity in 19 chilli landraces grown in kitchen gardens in different villages in comparison to the prevalent cultivar DKC-8 in a RCBD layout. Various characteristics were evaluated during both summer and winter seasons. It was observed that green fruit yield was affected during winter hence the selection based on green fruit yield should be done only during summer season. The observed variability in the landraces was just due to genotype but also environment effect in influencing variation. The traits such as green fruit yield per plant showed highest heritability and genetic advance among the tested landraces. The genotype CS9 was found to be early maturation and

CS18 recorded highest fruit weight (Fig. 1B). Overall, the evaluated chilli landraces were found to be highly pre-adapted to weather extremes and their valuable characteristics could be well utilized in future breeding programs (Fig. 1A) [24].

Among the various available plant breeding methods, mutation breeding has also been shown to have huge potential in generating genetically stable variations to improve crop yield and quality. Chemical mutagens may successfully alter a plant's genetic makeup, although the degree of success is highly dependent on the type and quantity of the chemical, and the kind of plant used in the experiment. Since a high mutagen concentration prevents seed germination due to loss of viability, breeders must identify the proper mutagen concentration without compromising seed viability. Recently, a mutation breeding programme in chilli was conducted for introducing genetic variation by treatment with chemical mutagens at ITM University, Gwalior. Sodium azide, Hydroxylamine mutagens at various concentrations have been tested for inducing mutations in the promising chilli cultivars namely, PusaJwala and PusaSadabahar and evaluated the traits such as germination, plant height and days to first flowering. The obtained data showed that sodium azide significantly affected the all evaluated traits, thus it exhibited higher potency in inducing genetic variability than hydroxylamine [25]. A recent comprehensive review on chilli breeding for industrial uses discusses the available information on various chilli bioactive compounds of industrial importance (capsaicinoids, capsinoids, carotenoids, phenolics and vitamins) and their estimation methods as well as industrial applications. In addition, it also comprehensively presented the available chilli genetic resources and molecular markers which could be used in future chilli breeding programmes [26].

Table 1: Chilli genotypes evaluated recently and the notable findings

Chilli Genotypes	Findings
Genotype 2	High fruit yield per plant [11]
Genotype 13	High green fruit weight [11]
P1 x P6	High specific combining ability [12]
CH-15140	High fresh fruit yield [13]
Bash Ghala	High fruit weight [14]
JAL x BIQ	Heterosis for yield and fruit morphology [15]
HAB x JAL	Heterosis for Capsacinoid content [16]
İnan3363 x PI 159236	Superior heterosis [17]
ByadgiDabbi	High fruit yield per plant [18]

ACS-08-09	High dry fruit yield [19]
ACS-18-08	Early maturity [19]
Plant number 91	High dry fruit yield [20]
IC-347044	High fruit yield per plant [21]
Grossum	High fruit weight [23]
CS9	Early maturity [24]
CS18	High fruit weight [24]
PusaSadabahar mutant	Reduced Plant height [25]

4. MOLECULAR STUDIES IN CHILLI

Simple sequence repeat (SSR), a microsatellite DNA is the widely used ~~PCR ~~PCR-based marker for assessment of genetic diversity among the genotypes. SSR markers are highly polymorphic, co-dominant, multi-allelic, abundant in the genome as well as practically highly reproducible, hence they are more helpful in analysis of genetic diversity and relatedness of the species than other available marker systems [27].

Recently, at the Plant Genetic Resources Centre (PGRC) of Bangladesh Agricultural Research Institute (BARI), Gazipur, a total of 10 summer growing chilli genotypes have been evaluated for their genetic diversity using SSR markers. Among ten chilli genotypes, eight local cultivars, one released variety (BARI morich-2) and one advanced line (SRC-517) were considered. Initially, 50 SSR marker primer pairs were tested to identify the polymorphism, and finally 8 SSR marker primer pairs (CAMS) were chosen for main analysis based on high level of polymorphism as well as their distribution on different chromosomes (Chr 2, 3, 5, 6, 7, 8). Based on the DNA bands (alleles) seen on the agarose gel, scoring was given and statistics of genetic variation was analyzed using various computer programs. In addition, Polymorphism information content (PIC) of each of the used SSR was also calculated (PIC= 1- ΣXi^{2}; Where, Xi is the frequency of the *i*-th allele of a particular locus). Data analysis revealed the existence of greater genetic diversity in the genotypes, which ranges from 0.45 (CAMS-885) to 0.74 (CAMS-647) as well as higher level of genetic differentiation (0.971) and lower level of gene flow value (0.007) further indicated the genetic diversity. Thus, this study provided insights into the genetic diversity among summer growing chilli genotypes grown in Bangladesh and recommended the utilization of these genotypes for future chilli breeding programmes for traits of interest [28]. Moreover, the same group of researchers also conducted SSR markers evaluation in 96 local cultivars and found out the existence of genetic diversity among the cultivars [29].

In another recent study conducted at KirsehirAhiEvran University, Turkey evaluated 69 chilli genotypes (56 local hybrids and 13 reference cultivars) for genetic variation using six SSR marker primer pairs linked to fruit morphology, and for the analysis of genes involved in capsaicinoids biosynthesis and resistance of *P. capsici*using SCAR (Sequence Characterized Amplified Region) markers. The results of this study showed the SSR markers such as CaeMS015 and CAMS452 were found to be highly polymorphic among the genotypes, and the SCAR marker BF6-BF8 was found to linked to pungency revealed the pungent genotypes whereas another SCAR marker, OP004.717 linked to *P. capsici* resistance revealed the resistance genotypes [30]. A recent review paper highlighted the significance of available multi-omics such as genomics, transcriptomics, metabolomics, proteomics, epigenomics, phenomics, speed breeding, agricultural robotics and panomics data on capsicum breeding and suggested the integration of these multi-omics would advance the understanding of the genetic basis for the various traits of the chilli and help quickly develop the chilli hybrids with the desired traits [31].

5. FUTURE PERSPECTIVES

The screening of chilligermplasm such as landraces and local cultivars would help choose the best parents with the great genetic variation for utilization in future chilli breeding programmes. The generation of interspecific chilli hybrids has been shown to have a great potential of attaining the genetic variation, thus resulting progeny would exhibit superior qualities than the individual species. Nonetheless, other technologies such as, marker assisted selection, genomic selection, genetic engineering by *Agrobacterium tumefaciens* mediated gene transfer, heterologous gene expression and genome editing method (CRISPR/Cas9) would also help develop chilli hybrids with desirable traits [32]. In addition, the future development of protocols specific to chilli crop for the latest breeding technology, the speed breeding would offer great advantages to evaluate many generations within less period of time and would help in quick selection of the best performing chilli hybrids [33].

6. CONCLUSION

In the current review, we discussed the recent achievements of the various research groups in the development of chilli hybrids with high yield and quality. Most of the studies majorly focused on analysis of genetic diversity among the generated chilli hybrids, local cultivars and landraces in order to select the best population with the high genetic variation for the future chilli breeding programmes. Some of the studies focused on the development of hybrids with a specific level of capsaicinoids content to control the fruit pungency, which is another interesting objective in chilli breeding. The application of marker assisted selection for selection of superior hybrids is also discussed. Moreover, the further utilization of natural polymorphism in chilligermplasm in combination with latest technological developments would be helpful in achieving a great success in the future chilli crop improvement.

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