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Molecular mapping of flowering traits revealed a major QTL, qDFFF3.3.1 associated with early maturity in cucumber (Cucumis sativus L.)

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Abstract

Background

Cucumber is an important cucurbitaceous vegetable with high demand in both domestic and international markets. It is primarily cultivated as a summer crop in tropical and subtropical regions. This study focused on flowering-related traits, such as early pistillate flowering and lower nodes for female flower initiation, which are desirable traits in breeding cucumber varieties and hybrids worldwide.

Results

A mapping population exhibiting considerable phenotypic variation was developed using two contrasting cucumber parental lines—DC-83 (early flowering) and DC-48 (late flowering)—selected for their differences in flowering time and shelf-life traits. Significant positive correlations were observed among flowering, shelf-life, and yield-related characteristics. Out of 1,200 SSR primers spanning all seven cucumber linkage groups, 106 polymorphic primers were selected based on a genome-wide polymorphism screening of the parental lines. These primers were then used for genotyping 125 individuals from the F_2 mapping population derived from DC-83 and DC-48. A comparative analysis of phenotypic and genotypic data led to the identification and mapping of four quantitative trait loci (QTLs) associated with days to first female flower (DFFF) and node to first female flower (NFFF). These QTLs were located on chromosome 2 (qNFFF2.1), chromosome 3 (qDFFF3.3.1 and qDFFF3.1), and chromosome 7 (qDFFF7.3.2). This study represents the first report of QTLs linked to DFFF and NFFF traits in cucumber genotypes of Indian origin, where a broad range of trait diversity is present. Notably, most of the identified QTLs exhibited higher phenotypic variance explained (PVE) percentages compared to earlier reports: qDFFF3.3.1 (35.14%), qDFFF3.1 (27.18%), qNFFF2.1 (6.95%), and qDFFF7.3.2 (4.7%).

Conclusion

Overall, the results from this study including phenotyping, SSR marker screening, QTL mapping, and flanking marker locations offer a strong scientific foundation to support cucumber improvement programs. These advancements include marker-assisted selection (MAS), genomic research, diversity analysis, DNA fingerprinting and genetic mapping for yield-related traits, facilitating the development of high-yielding cucumber varieties and hybrids.

Background

The cucumber, scientifically known as *Cucumis sativus L.*, is a diploid plant with 2n=2x=14 chromosomes. Commonly called *kankri* and *khira* in Hindi, cucumber ranks fourth most important vegetable after tomato, onion and cabbage. Based on literatures cucumber likely originated in India, possibly in the Himalayan foothills, where many wild and cultivated species are widely available. The Cucurbitaceae family is considered monophyletic due to its lack of close genetic relatives. Major groups within Cucurbitaceae include cucumbers, squashes, melons, pumpkins, and watermelons [1,2]. The *Cucumis* genus includes cultivated species like cucumber (*C. sativus*) and melon (*C. melo*), as well as non-traditional and pre-breeding usable species such as *C. sativus var. hardwickii*, *C. callosus, C. hystrix* and *C. muricatus,* whichmost likely utilized in disease resistance breeding for downey and powdery mildew diseases as well as various economic traits, including parthenocarpy, gynoecy, flowering-related traits and yield-attributing characteristics have been studied and mapped in over 50 identified *Cucumis* species, which display unique phenotypic and genetic diversity across Africa, Asia and India. *Cucumis sativus var. hardwickii* is the wild ancestor species of the currently cultivated cucumber. Mostly available at higher elevations with unique characters like resistance to fungal disease, crown fruit set initiation, tiny fruit with pubescence on stem and flower parts [3]. *Chinese long' 9930* an inbred line, utilised for genome sequencing in cucumber (367 Mb) [4].

This crop is both frost-sensitive and thermophilic, with an optimal growth and development temperature of 27 ±2 °C. Consequently, it is primarily cultivated in tropical to subtropical regions with only limited cultivation in temperate areas [5]. The plants have a vining growth habit, reaching 2 to 2.5 meters in length, with extensive branching. The male flowers are light to dark yellow and appear in clusters, while the female flowers are solitary with inferior ovaries [6]. Cucumber is regarded as a nutritious fruit, primarily cultivated during the summer season. In a 100 g serving, cucumber contains 96.3 g of moisture, 0.4 g of protein, 0.3 g of minerals, 0.4 g of crude fiber, 0.1 g of fat, and 2.5 g of carbohydrates. It also provides 10 mg of (Ca) calcium, 25 mg of phosphorus (P), 0.60 mg of iron (Fe), and yields 13 Kcal of energy [7-9], which aids in weight loss, promotes glowing skin, regulates blood pressure, supports body hydration and enhances digestion [10-11]. Currently, cucumber and gherkins are grown on approximately 198,518 hectares globally, with a production of 75,219,440 tonnes and a productivity of 37.90 tonnes per hectare [12]. Among top producers of cucumber, India ranks 26th ranking after China, Turkey and Russia [13], According to NHB data from 2022, cucumber cultivation in India covers nearly 109,000 hectares, with a national production of 1,696,000 tonnes and a productivity of 15.56 tonnes per hectare.

Cucurbits possess unique flowering characteristics. In cucumber, the anthesis period and flower distribution pattern differ from other vegetable crops and can be categorized into three distinct sexual phases: (i) an early androecious phase, where only male flowers appear from the 1st to the 7th node; (ii) a mixed phase, where both male and female flowers emerge simultaneously between the 7th and 12th nodes; and (iii) a late or predominantly female phase, where female flowers are more frequent from 12th node onwards. These flowering patterns are strongly influenced by environmental factors and hormonal activity [14]. In cultivated cucurbits, monoecious plants, which have both staminate and pistillate flowers on the same plant, are the predominant sex form. Other forms include dioecious plants, with male and female flowers on separate plants, and hermaphroditic flowers, where both staminate and pistillate flowers reproductive organs are located within a same flower. The gynoecious sex form is the most economically significant and is widely used in breeding high-yielding, parthenocarpy cucumber hybrids for protected cultivation worldwide [2]. Sex expression and differentiation was controlled by genetic, hormonal and

environmental factors. Three key genes solely controlling the sex expression in cucumber: (*F* locus) female, (*A* locus) androecious,(*M* locus) monoecious. Partially dominance and catalytic to early induce of female phase in cucumber. Gynoecious sex expression is controlled by *FF* locus. *A* locus and *M* locus are taking responsive expression on the key traits like maleness and inhibition effects on male/stamen development respectively [15-17].

In cucumber, genomic locations taken key role in expression and induction of sex forms such as *Androecious* (*A*),*Gynoecious* (*F*) and *Monoecious* (*M*). The '*F'* locus represented partial dominance to control gynoecious character and stimulates to induce of the female phase early, while *FF* loci containing cucumber plants are gynoecious. '*A'* locus is responsible for staminate flowers, while the *M* locus dictates the inhibition of stamen development [16,17]. The key enzymes taken part in ethylene biosynthesis (ET) was *ACC* synthases which encoded by the major genes such as *F* and *M* genes. *F* locus (*CsACS1G*), which is also having duplicated form named as *CsACS1* present as a single copy, controls monoecious sex expression [18,19, 20]. External hormonal applications, such as auxins, GA_3, and brassinosteroids, can enhance growth by reducing internodal length and increasing the number of female flowers per cucumber plant. GA_3 is known to have a masculinizing effect on cucurbits. Mutations in various genes involved in ethylene biosynthesis can alter flowering patterns. In cucumbers and melons, the *ACS11* and *ACO2* genes play a significant role in carpel development. *CsACS2* of cucumber, inhibition of stamens in female flower [21,22].

Considering the complexity of quantitative traits such as days to 1st pistillate flowering (DFFF), node to 1st pistillate flower initiation (NFFF) and other yield-related traits, research continues to progress from traditional breeding approaches to advanced genomics-based breeding methods on different cucumber materials. Flowering period/time, which is key trait for plant life cycle and major role in all other productivity related traits. Vegetative phase to reproductive phase marked by the flowering initiation and environmental stimuli like photoperiod and mean temperature during flowering phase. Early flowering at initial nodes of vine is major breeding objective in all the cucurbits. For avoiding major environmental stresses and threats during late flowering phases, such as diseases like powdery mildew, downy mildew, and anthracnose, along with stresses from cold and high temperatures, is crucial as these factors disrupt flowering sequences and reduce plant productivity. Therefore, genotypes that produce early pistillate flowers on the initial nodes of vine growth are highly beneficial for cucurbit breeding. Building on this knowledge and available resources, numerous cucurbit breeders have investigated both traditional genetics and advanced approaches, including QTL analysis and fine mapping. Mapping agronomic traits such as the number of lateral branches, the number of fruits per plant, and the days to staminate flower initiation has been conducted using mapping populations like $F_{2:3}$ and RILs [23, 24]. QTLs for powdery mildew resistance [25]. Flowering traits like female flower ratio (*sex6.1, sex2.1, sex2.2,*), first female flower node (*fffn5.2, fffn2.1, fffn2.2, fffn1.1, fffn3.1, fffn5.1,*) were mapped [26]. Other traits such as fruit shape, parthenocarpy flower sex were also mapped and recorded [27-30]. Major QTL, *Parth6.1*, linked to parthenocarpic trait in PPC6 a slicing cucumber genotype was mapped and parthenocarpy fruit set traits [31,32]. Following whole genome re-sequencing method major candidate gene for parthenocarpic QTL (*Parth 2.1*) trait was mapped [33].

Flower traits are essential in cucumber breeding. The ratio of pistillate flowers and the node of the first female flower significantly impact the number of fruits per plant, thereby affecting overall yield. Keeping view on previous research gaps, and studying flowering related QTLs on different breeding materials were great scope in the marker assisted selection and development of early verities and hybrids, with this content our study primarily focused on key traits, such as days to 1st pistillate flower (DFFF) and node to 1st female flower initiation (NFFF), within our F_2 breeding population. This population was generated from a cross between two contrasting parents for flowering traits: DC-48 (late flowering with distant flowering nodes) and DC-83 (early flowering at early nodes). The populations was genotyped with SSR markers and genetic mapping for days to first pistillate flowering (DFFF) and node to first pistillate flower initiation (NFFF) were conducted.

Results

Phenotypic variations and correlation coefficient analysis for flowering traits in F_2 mapping population

ANOVA (analysis of variance) studies depicting that statistically significant differences for two polygenic traits related to flowering (Table. 4 and 5). The parent lines DC-83 and DC-48, used to create F_2 and $F_{2:3}$ mapping populations, showed contrasting flowering characteristics. DC-83 exhibited fewer days to first pistillate flower (DFFF) and a lower node to first pistillate flower (NFFF) in contrast, DC-48 took 5-10 additional days for flowering and had a more distant node for the initiation of flowering compared to DC-83. Wide ranges, maximum and minimum values of two flowering related phenotypic traits for both summer (2022 and 2023) and kharif (2022) seasons was presented the maximum variability in the $F_{2:3}$ mapping population (Table. 2). The data shown that range for days to first female flowering varied from 39.45 days (summer season -2022) to 54.40 days (kharif season -2022) with the mean value 44.30. Earliness in female flowering is considered to be a favourable character with respect to yield and also to avoid different biotic and abiotic stresses during the later stage of crop. The number of nodes presenting the population varied significantly among $F_{2:3}$ populations. The number of nodes at which the first female flower appears varies between 3.39 (summer season -2022) and 4.61 (summer season -2022). There was differential flowering pattern in the $F_{2:3}$ population due its parental genetic makeup (Fig.1 (B) (E)). The segregation pattern for the data points of the two selected flowering traits is represented in box plots graphs. Across the three seasons, some deviations in the data indicate the presence of transgressive segregants within the selected mapping population. Days to first female flowering across the three-season having some sort of segregation distortion due to the occurrence of transgressive segregates in $F_{2:3}$ population or the different seasons with different mapping populations sets used in the phenotypic evaluation studies. The box plot (Fig. 5 (A) (B)), organized into continuous classes, illustrate that both traits are governed by polygenes or quantitative genes. According to the data points, outliers are minimal, indicating that the phenotypic data follows a normal distribution. GCV, PCV, (h^2) Heritability and (GA) Genetic Advance, GAM for two phenotypic traits in cucumber for flowering related traits were statistically analysed (Table. 3). The larger number of class intervals further suggests the presence of contrasting traits within the mapping populations across the three seasons of study (Fig. 1).

Parental polymorphism and hybridity testing

To construct a linkage map, a polymorphism survey was conducted on the two parental inbreds using SSR markers that are evenly distributed across the seven linkage groups. Approximately 1,200 SSR markers were screened on the parental lines, and 106 primers were selected based on polymorphism and Mendelian allele distribution patterns (Fig. 2 (A)). Various polymorphic primers were identified due to size differences in the amplicons, with most amplified products ranging from 150 to 250 bp. The confirmed polymorphic primers were then used for hybridity testing and genotyping of F_2 populations (125 progenies) (Fig. 2 (B)).

Linakge maps construction and *QTL* analysis

Genotyping on 125 F_2 mapping population total of 106 polymorphic SSR primers (Supplementary Table. 1) were utilized. The phenotypic data collected from the three seasons (summer season -2022 & 2023 and kharif season -2022) were also utilised in QTL analysis. Linkage maps with QTLs mapped as well as the flanking markers linked to DFFF and NFFF traits are generated based the three environmental conditions. The outcomes generated from the results of SMA-single marker analysis and CIM- Composite Interval Mapping, overall four QTLs were detected for the flowering trait in (F_2: DC-83 x DC-48) mapping populations. Among the four detected QTLs, two major and two minor QTLs were identified.

QTL mapping for DFFF

Days to first female flower is one of the most important traits in all the cucurbits with respect to the earliness. Two genomic regions from chromosome 3 for DFFF (*q DFFF3.3.1, q DFFF3.1*), one QTL on Chromosome 7 (*q DFFF7.3.2*) were found to have influence and consistency on the days of the first female flower in cucumber. However, consistency was majorly observed two QTLs (*q DFFF3.3.1, q DFFF3.1*) on chromosome No.3, with a LOD value of 10.46 and 7.71 and additive effect of -0.98 to – 3.07 was detected during summer and rainy season -2022. Node to first female flower is an economic trait in cucumber for its early harvest and yield enhancement (Table. 1; Fig. 3 (B) (C)).

QTL mapping for NFFF

Node to 1st pistillate flower anthesis is an important quantitative trait influence by environmental and genomic make up. Both the parents differed significantly differs for this trait, node to 1st pistillate flower anthesis. Similarly, the F_2 population also showed a wider variation for node to 1st pistillate flower anthesis viz., early node to later nodes of flower initiation. In this study, one QTL (*q NFFF2.1)* was identified for node to 1st pistillate flower anthesis during summer seasons of 2022, *QTL* detected on the Chr. 2 QTL (*q NFFF2.1*) found to be minor with PVE % was 6.95 % during summer season -2022. Combined linkage map for all three chromosomes respective LOD scores and additive effects of the detected QTLs were tabulated (Table. 1; Fig. 3 (1))

Discussion

Cucumber is a major salad vegetable with high demand in both domestic and international markets. However, it faces significant challenges, including susceptibility to environmental and biotic stresses. Due to its inherent genetic characteristics, cucumber typically produces male flowers before female (pistillate) flowers, leading to delayed harvest times and fluctuating market prices. This trait also increases the crop's vulnerability to major fungal and bacterial diseases. In current breeding programs, early pistillate flowering and lower nodes for female flower initiation are desirable traits in cucumber varieties and hybrids worldwide. Thus, there is a pressing need to explore unique cucumber genotypes and investigate the phenotypic and genetic characteristics related to early flowering. In line with current breeding objectives, our mapping experiments have identified several major and minor QTLs (*qDFFF3.3.1, qDFFF3.1, qNFFF2.1, qDFFF7.3.2*) associated with flowering traits in F_2 mapping populations having the LOD value of 10.46 and 7.71 and additive effect of -0.98 to – 3.07 for DFFF and *QTL* detected on the Chr. 2 (*q NFFF2.1*) found to be minor with PVE % was 6.95 % during summer season -2022. Combined linkage map for all three chromosomes respective LOD scores and additive effects of the detected QTLs were represented (**Fig. 4**). These findings provide valuable insights for further research on flowering traits within cucumber improvement programs. Unfortunately, few studies have focused on QTL mapping for flowering traits in cucumber, primarily due to limited availability of quality genetic resources [34]. Till date few mapping experiments were recorded on cucumber flowering traits such as the days to 1st pistillate flower anthesis (DFFF) and a lower node to 1st pistillate flower anthesis (NFFF) in cucumber. Genetic mapping of and identification of the QTLs related, female flower ratio (*sex2.2, sex2.1, sex6.1*), first female flower node (*fffn5.2, fffn1.1, , fffn2.2, fffn3.1, fffn2.1 fffn5.1,*) were mapped [34]. QTL flagged for fruit agronomic traits using F_3 population was published [35,24]. Mapping for number of fruits per plant and primary, days to female and male flower anthesis and lateral branching number was successfully mapped using F_3 families [23]. In many horticultural crops mapped QTLs outcomes such as QTL loci, QTL number and phenotypic variance are greatly influenced by the mapping populations nature and size, environmental influence as well as the trait under selection [36,37,25,30].

SSR or are frequently favoured for QTL mapping due to their co-dominant inheritance, reliability, widespread distribution within the genome and multi-allelic/multi loci mapping properties [38, 39]. In this molecular mapping experiment, microsatellite markers were chosen to develop a linkage groups with respective QTls flagging and to identify QTLs related to early and late flowering traits and extended life shelf-life relative genomic regions in cucumber. The cucumber genome has been completely sequenced (ftp://ftp.ensemblgenomes.org/pub/plants/release-47/fasta/cucumis_sativus/dna/.

) [4], offering an extensive selection of SSR markers for QTL mapping. Identifying quantitative trait loci (QTL) in mapping populations provides the advantage of directly applying this knowledge to enhance crops through informed, knowledge-based breeding strategies. Identification of QTLs in plants was performed utilizing the genotyping data and screened phenotypic variances of a quantitative/polygenic trait in a segregating generation such as F_2 (preliminary mapping population) resulted from the crossing of two parental inbred lines (DC-48 and DC -83) in cross-pollinated crops [40,41].

In the current investigation aimed at mapping the flowering trait in cucumber, 125 F_2 populations were selected for genotyping (106 SSR polymorphic primers), phenotyping (Two summer season 2022 & 2023, kharif season -2023) and subsequent population advancement up to RILs F_7 generation. This

population size is comparable to those used in other vegetable crop studies involving F_2 populations, QTL mapping for anthracnose resistance in Chili pepper 126 F_2 mapping population [42], comparative mapping studies in interspecific chilli line 126 F_8 RILs mapping population [43]. Yield-related quantitative traits mapping in Tomato, 160 F_2 population [43]. However, unlike most of the earlier studies, in this study, an F_2 mapping population was utilized, which provided a broader range of phenotypic variation for the traits of interest. This diversity facilitated the identification of significant QTLs with substantial effects, supporting their potential application in marker-assisted selection.

Conclusions

A mapping population with high phenotypic variability was developed using two cucumber parental lines, DC-83 and DC-48. Statistically significant positive correlations were observed for yield-related traits. From 1,200 SSR primers spanning all seven cucumber linkage groups, 106 polymorphic SSR primers were selected after screening for polymorphism at the genomic level in the two parental lines. These primers were then used for genotyping studies on 125 F_2 mapping populations derived from DC-83 and DC-48. Comparative phenotypic and genotypic analysis identified and mapped four QTLs (*qDFFF3.3.1, qDFFF3.1, qNFFF2.1, qDFFF7.3.2*) associated with days to first pistillate flower (DFFF) and the node to first pistillate flower (NFFF) in cucumber. This study marks the first documented exploration of DFFF and NFFF traits in Indian-origin genotypes, where a rich diversity of traits is available. The majority of QTLs mapped showed statistically significant LOD scores and PVE percentages when compared to previous studies. Overall, the findings from this research, including phenotyping, SSR marker screening, QTL mapping, and flanking marker locations, provide scientific evidence that can accelerate cucumber improvement programs. These include marker-assisted selection (MAS), genomic studies, diversity assessment, DNA fingerprinting, and genetic mapping for yield-related traits, ultimately aiding in the breeding of high-yielding cucumber varieties and hybrids.

Materials and methods

Development of F_2 and $F_{2:3}$ mapping population

The planting genetic resources include two inbred parental lines: DC-83 (Pusa Long Green), which is early flowering and matures quickly, with the first harvest occurring 45-50 days after planting. This line, classified as a slicing-type cucumber, is designated as Parent 1 (P1). Parent 2 (P2) is the inbred line DC-48, a natural variant with an extended shelf life trait, which flowers and matures later at 55-60 days after sowing. Their F_1 (DC-83 × DC-48), F_2 (selfed F_1) and $F_{2:3}$ population generated from individual plants selfed/advanced from the F_2 individual progenies (Fig. 1(A)). Seeds of both parental lines were obtained from the Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi (28.6377° N, 77.1571° E). The F_1 hybrid from the cross DC-83 × DC-48 was developed during the summer season (April-August), 2019-20, in an insect-proof, netted polyhouse (Fig. 2). DNA was isolated from the F_1 population for two parental genetic polymorphism screening and hybridity identification using simple sequence repeat (SSR) markers marker from cucumber genome. F_2 progenies were developed during the Rabi season (November-February) of 2019-20, selected from hybridity-confirmed F_1 seeds (Fig. 1 (F)). A total of 125 seeds from a single selfed F_1 plant were used to produce the F_2 progenies. Each of these 125 F_2 progeny plants was selfed individually to advance to the $F_{2:3}$ population. Similarly, $F_{6:7}$ RILs were generated by individual plant selfing from the $F_{2:3}$ population to get pure homozygous inbred lines. F_2 progenies were used for genotyping studies and the 125 $F_{2:3}$ used for phenotyping studies for 3 subsequent seasons for 3 traits associated with extended shelf-life. During population development and advancement, recommended plant growth boosting fertilizers and culture practices were timely maintained along with the plant production and protection measures to get healthy and disease-free plants.

Phenotyping data collection

Under open-field conditions, two parental lines (DC-83 and DC-48) and their 125 $F_{2:3}$ progenies (preliminarily advanced RILs) were evaluated across three seasons: summer 2022, summer 2023, and kharif 2022. The study used a Randomized Block Design (RBD) with ten replications for each $F_{2:3}$ progeny to ensure robust data analysis. Trials were conducted at the Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi, India (28.6377° N, 77.1571° E), a region with a subtropical climate characterized by approximately 774.4 mm of annual rainfall and average maximum and minimum temperatures of 45°C and 1.5°C, respectively. The seeds for the 125 $F_{2:3}$ progenies were sown in raised channel beds with spacing of 0.75 meters between plants and 1.0 meter between rows. During the growing period, all recommended agronomic practices were consistently monitored. The data was recorded from 10 individual of 125 $F_{2:3}$ progenies (1250 plants) along with two parental lines (DC-83 and DC-48). A total of two flowering related quantitative traits (days to first female flowering (DFFF) and node to first female flowering (NFFF)) were recorded at fully developed plants which were transforming from vegetative phase to reproductive phase.

The two flowering related quantitative traits such as (i) Days to first female flowering (DFFF) and (ii) Node to first female flowering (NFFF) readings was recorded at 25-30 days after sowing (Fig. 1(B) (C) (D) (E)). Cucumber anthesis periods varies from 7.30 am to 9.30 am based on the environmental and genetic architecture of the genotypes, accordingly DFFF and NFFF were recorded during early morning hours in research filed. Similarly, the total number of nodes and days to first pistilate flower initiation up to the complete life cycle of the cucumber to record accurate data.

Phenotypic data analysis

The mean data on NFFF and DFFF of the two parental lines and the descriptive statistics such as mean, maximum, minimum, range, variance, skewness and kurtosis of the mapping population was analysed using the SPSS 16.0 and Microsoft Excel. Pearson's correlation coefficient and box plots for these two quantitative traits were analysed described in R version of 4.2.4 under three different seasons (summer 2022, summer 2023, and kharif 2022). Histograms with normal distribution curve was generated (frequency distribution with bar chat) for genetic analysis and inheritance studies. Analysis of variance

(ANOVA) were did for the collected data on two flowering traits using Randomized Block Design (RBD) [44]. (Genotypic coefficient of variation-GCV, Phenotypic coefficient of variation - PCV), h^2- heritability), Genetic advance mean value - GAM were statistically analysed using the formulae [45, 46, 47].

$$GCV\ (\%) = \sqrt{\sigma q^2} / \ \bar{x}$$

$$PCV\ (\%) = \sqrt{\sigma p^2} / \ \bar{x}$$

$$h^2 = \sigma q2/(\sigma q^2 + \ \sigma e^2)$$

$$GAM = \ (GA./X)\ x100$$

where, $\bar{x}$ is mean values of the samples, σg^2, σe^2 and σp^2 are the components of variance due to genotypes, errors an phenotypes, respectively. h^2 was expressed in percentage.

Genotyping

Genomic DNA isolation, quantification and PCR amplification

Parental and mapping population genomic DNA was isolated from healthy, young shoot leaves of the cucumber (Doyle and Doyle, 1990). Using a Nano Drop 8000 spectrophotometer (Thermo Fisher Scientific) the contaminations and concentration of isolated genomic DNA was quantified. Where, A260:A280 ratio showing above 1.8 (Protein/amino acids contamination), 1.4 to 1.7 (PCR usable DNA) and below 1.4 (RNA contamination). PCR amplification mixture containing, 10 µl final volume fortified with 1.5-2.0 µl genomic DNA (10 ng/ µl), 1.0-1.5 µl mixture of forward and reverse primer (50 ng/ µl). PCR master mix (GoTaq, Master Mixes-Promega's) (5.0 µl) and 2.0 µl of nuclease free doubled distilled water. For cucumber, PCR amplification optimized programme were performed using thermocycler (Master cycler nexus-Eppendorf India) as described by Dey et al., 2006. with initial denaturation at 95 ℃ for 5 min; followed by next cycle programme of 34 cycles amplification includes, 94°C, 1 min; 50-60 °C, 40s; 72°C, 40 seconds, lastly settled the PCR programme for infinite time at 4 °C. Amplified products were mixed well with PCR product before being dissolved on 3 percent agarose gels (in 1X TAE) with Tris/Acetate/EDTA (TAE) stained with ethidium bromide. The PCR substance and dye mixture were loaded into the well of a horizontal gel electrophoresis device (Bio-Rad, USA). In the side or middle well, a known DNA size marker was loaded for 3 hours, a steady voltage of 120 V was maintained. Stain the gel with ethidium bromide (0.33µg/ml) and then observe on UV-transilluminator. Judge the quality of DNA samples on the basis of whether sample DNA formed a single high molecular weight band or smear (Alpha Imager Pvt. Ltd., India).

Screening of SSR markers for polymorphism survey

A total of 1200 SSR (Simple sequence repeats) cucumber primers which are evenly distributed among all the seven linkage groups (Supplementary Table 1) were utilized for polymorphism survey on two parents (DC-83 and DC-48) to identification of polymorphic primers. The identified polymorphic primers of 106 SSR markers were used to genotyping studies on F_2 progenies resulted from two flowering related contrasting parents (DC-83 and DC-48). All the 106 SSR polymorphic primers showing the Mendelian segregation ratio (1:2:1) and hence those were ideal for genotypic survey on the segregating population (F_2). For statistical analysis, amplified SSR marker information were visually scored as A (Parent -1), B (Parent -2) and H for Heterozygous (F_1). If the small band (DNA strip) size of F_2 progeny was equal to Parent -1 (DC-83), then it considered as 'A' score. Similar way, if the DNA band is equal to parent -2 (DC-48), 'B' score was assigned. If the DNA bands having two stripes (heterozygous state) with each band size equal as parents, then it was considered as 'H' score.

Linkage mapping and *QTL* analysis

The ratio segregation ratio's of all the SSR polymorphic markers was analyzed chi-square test (chi-square test), to confirmation and test the goodness of fit for Mendelian segregation (1:2:1). Seven linkage maps were constructed using 106 polymorphic SSR markers between DC-83 and DC-48. The genetic distances (Centi-Morgans) were calculated using the Kosambi mapping function. *QTL Icimapping software v4.2* [48], was employed to map the identified QTLs associated with two flowering-related traits, DFFF and NFFF, within the respective linkage groups, with a systematic naming convention for the QTLs with a LOD score (minimum logarithm of odds) 2.50. Single marker analysis and Inclusive Composite Interval Mapping Additive (ICIM-ADD) theory of *IciMapping v 4.2* was followed to survey the QTL using the seven linkage map and phenotypic observations. The QTL nomenclature for each linkage group is denoted as, for example, *qDFFF3.3.1*. Here, "*qDFFF*" represents the QTL for days to 1st pistillate flower anthesis, while "3.3.1" specifies, "3" refers to chromosome/linkage group 3, the second "3" indicates the season, and "1" represents the number of *QTLs* identified for particular trait within the same season.

Declarations

Ethics approval and consent to participate

Not applicable.

Authors contributions

PKN, CP, SSD initiated and designed the research. PKN and SSD wrote the main manuscript text. PKN and SSD performed the experiments. PKN,NK,RB,SSD analyzed the data. BRC, AK, BST, ADM, TKB revised the paper. All authors rea and reviewed the final manuscript.

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Data availability

The data sets supporting the results of this article are included in the supplementary file.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Tables

Table 1. Stable QTLs detected for cucumber (*Cucumis sativus*. L.) flowering related traits in the DC-83 x DC-48: F_2 and $F_{2:3}$ mapping population.

Sl. No	Trait Name	QTL name [f]	Chromosome [a]	Position	Left Marker	Right Marker	LOD [b]	PVE (%) [c]	Add [d]	Dom [e]	LeftCI	Right(
1	DFFF_Kharif 1	*q DFFF3.3.1*	**3**	202	SSR03918	SSR01573	10.4635	35.1405	-3.0764	-1.3121	191.5	212.
2	DFFF_Kharif 1	*q DFFF7.3.2*	**7**	423	SSR11741	NR56	2.7197	4.7005	-0.981	-0.9118	387.5	423
3	DFFF_ Summer 1	*q DFFF3.1*	**3**	165	SSR03918	SSR01573	7.7155	27.1854	-1.8846	-2.743	157.5	176.
4	NFFF_Summer 2	*q NFFF2.1*	**2**	1158	UW036913	MU12359_1	2.5086	6.9563	0.9136	-1.0739	1141	118

Note: Unconditional consensus QTLs underlying the DFFF,NFFF and FF, FFtop, FFmiddle, FFbottom, RGC and FS traits at field and lab screening for extended shelf-life in Cucumber. **a** Chromosome. **b.** The 2.5-LOD confidence interval of QTLs. **c.** The phenotypic variance (PVE%) explained by each QTL (<10 for minor and > 10 major QTLs. **d.** Additive effect **e.** Dominant effect (-ve sigh Add and Dom for male parents' contribution)**. f.** *QTL* manes.

Table 2. Descriptive statistics for the flowering related traits in cucumber (*Cucumis sativus*. L) DC-83 X DC-48 derived $F_{2:3}$ mapping population.

Traits	Generation	Season	Minimum	Maximum	Mean ± Std Dev	Range	Variance	CV%	Skewness	Kurtosis
DFFF	$F_{2:3}$	S1	39.50	50.68	44.30±2.07	11.1	4.29	10.38	0.08	0.98
		S2	39.45	54.40	45.27±2.54	14.95	6.45	5.88	1.01	1.79
		S3	39.66	53.97	45.14±2.30	14.31	5.33	5.89	0.84	1.32
NFFF	$F_{2:3}$	S1	3.39	4.61	3.68±0.17	1.22	0.03	43.58	1.46	4.86
		S2	3.56	4.35	3.75±0.11	0.78	0.01	42.20	1.60	5.72
		S3	3.51	4.49	3.74±0.13	0.97	0.01	41.79	1.61	5.73

Note: DFFF – Days to first female flower, **NFFF**- Node to first female flower, **RGC**- Retention of green color, **FS**- Fruit shrinkage, **FF**- Fruit firmness, **TopFF**- Fruit firmness at top, **MiddleFF**- Fruit firmness at middle, **BottomFF**- Fruit firmness at bottom. S1 (Season-1), S2 (Seson-2), S3 (Season-3). F2:3 mapping population. Skewness (-ve) – values distributed left, Kurtosis (-ve)- a shape flatter than normal

Table 3. GCV, PCV, (h^2) Heritability and (GA) Genetic Advance, GAM for flowering related traits.

Genetic parameter	Summer season 1		Kharif season 1		Summer season 2	
	DFFF	**NFFF**	**DFFF**	**NFFF**	**DFFF**	**NFFF**
GCV	6.85:LOW	12.72:MODERATE	6.62:LOW	9.70:LOW	6.17:LOW	10.83:MODERATE
PCV	12.43:MODERATE	45.39:HIGH	8.85:LOW	43.29:HIGH	8.53:LOW	43.17:HIGH
h^2	30.36:MODERATE	7.85:LOW	55.95:MODERATE	5.02:LOW	52.31:MODERATE	6.29:LOW
GA	3.44:LOW	0.27:LOW	4.62:LOW	0.16:LOW	4.15:LOW	0.20:LOW
GAM	7.77:LOW	7.34:LOW	10.21:MODERATE	4.47:LOW	9.19:LOW	5.59:LOW

Note: DFFF – Days to first female flower, **NFFF**- Node to first female flower, **RGC**- Retention of green color, **FS**- Fruit shrinkage, **FF**- Fruit firmness, **TopFF**- Fruit firmness at top, **MiddleFF**- Fruit firmness at middle, **BottomFF**- Fruit firmness at bottom. **PCV** = Phenotypic coefficient of variation, **GCV** = Genotypic coefficient of variation, estimates of **PCV** and **GCV** were classified as low (< 10%), moderate (10%-20%) and high (>20%). h^2 = Heritability in broad sense, classified into three groups as low (< 50%), moderate (50%-70%) and high (>70%). **GA** = Genetic Advance and Genetic advance as percentage of mean (**GAM**) was categorized as low (< 10%), moderate (10%-20%) and high (>20%).

Table 4. Analysis of variance (ANOVA) for days to first female flower in the DC-83 X DC-48 derived $F_{2:3}$ mapping population.

	Summer season -1		Kharif season -1		Summer season -3	
Source	F-Value	Pr (>F)	F-Value	Pr (>F)	F-Value	Pr (>F)
Replication	12.24***	0.0006	2.98	0.0868	3.04	0.0837
Genotype	1.87***	0.0003	3.54***	0.0000	3.19***	0.0000
Mean	44.31		45.27		45.15	
Std Dev	5.59		4.01		3.86	
CV (%)	10.38		5.88		5.89	
CD	6.41		6.41		6.41	

Note: * and *** indicates, the $p \leq$ values were significantly different at the 0.05 and 0.01 probability level.

Table 5. Analysis of variance (ANOVA) for node to first female flower in the DC-83 X DC-48 derived $F_{2:3}$ mapping population.

	Summer season -1		Kharif season -1		Summer season -3	
Source	F-Value	Pr (>F)	F-Value	Pr (>F)	F-Value	Pr (>F)
Replication	5.12*	0.02	2.86	0.0934	3.40	0.0674
Genotype	1.17	0.19	1.11	0.2883	1.13	0.2418
Mean	3.69		3.75		3.74	
Std Dev	1.69		1.69		1.62	
CV (%)	43.58		42.20		41.79	
CD	6.41		6.41		6.41	

Note: * and *** indicates, the $p \leq$ values were significantly different at the 0.05 and 0.01 probability level

Figures

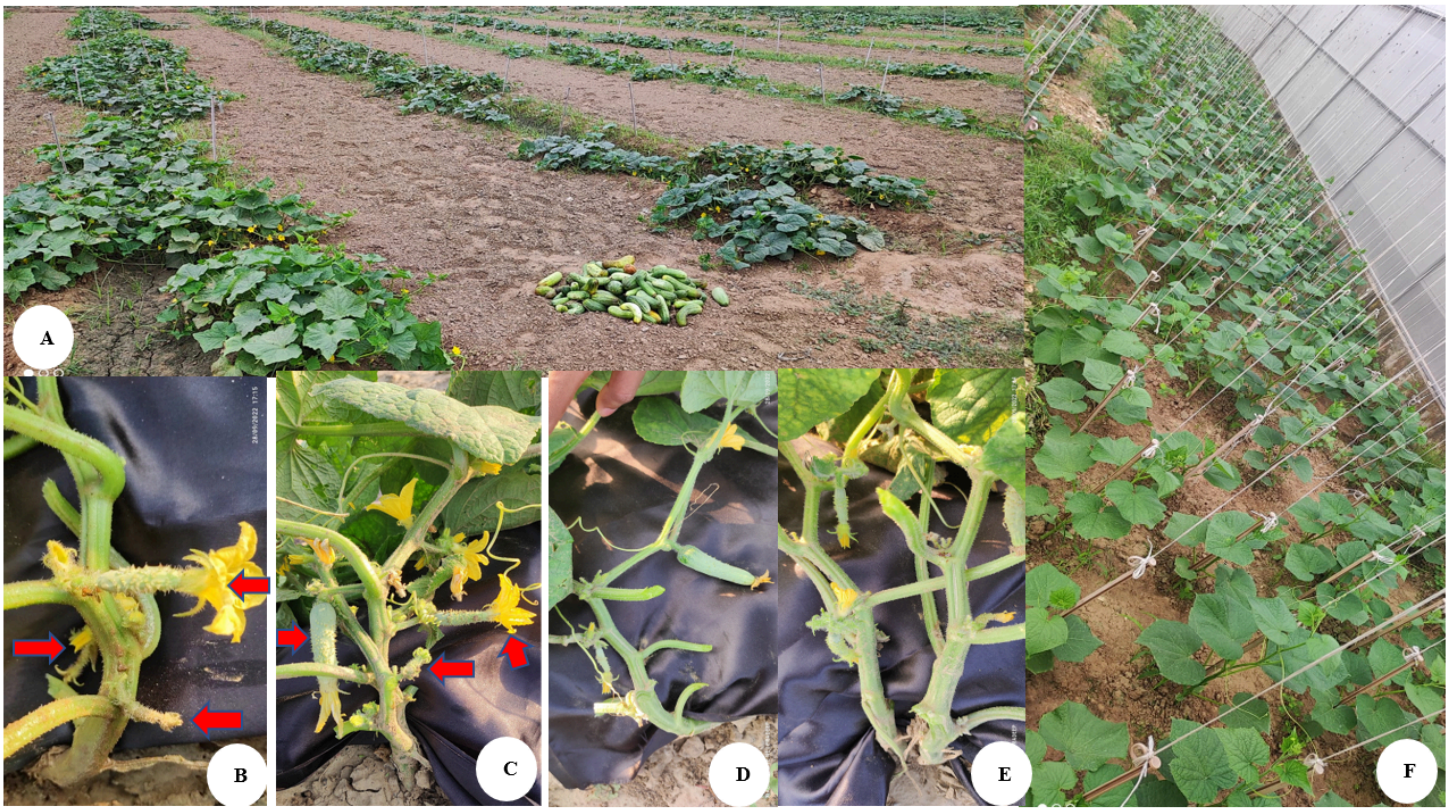

Figure 1

(A) Evaluation of the $F_{2:3}$ cucumber mapping population derived from the cross DC-83 × DC-48 under open field conditions across three seasons (summer and rainy seasons of 2022 and summer season of 2023). Abundant fruiting observed in $F_{2:3}$ population plants derived from the cross DC-83 × DC-48, grown under open field conditions and evaluated for (**B and C**) Days to First Female Flowering (DFFF) and (**D and E**) Node to First Female Flowering (NFFF). **(F)** F_1 seed production in insect proof net house condition

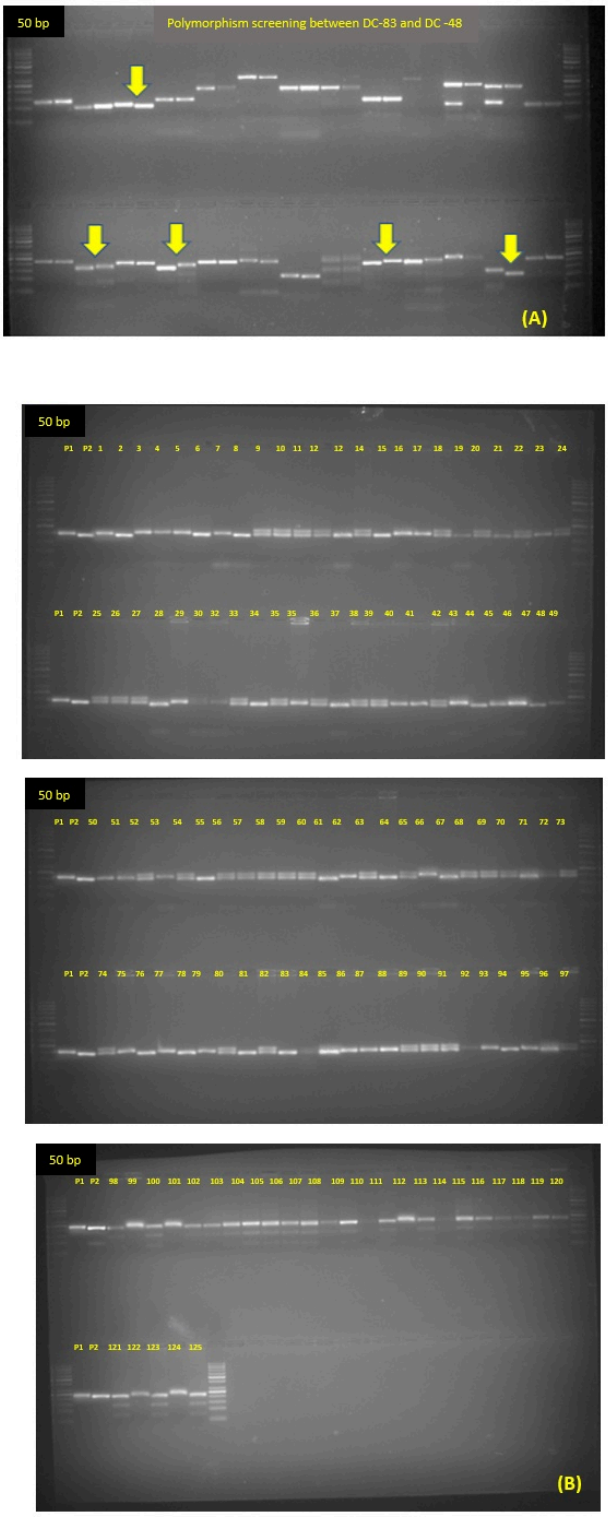

Figure 2

(A). Polymorphism survey by using 1200 SSR markers on DC-83 and DC- 48. **(B)** Genotyping of 125 F_2 mapping population derived from the cross of DC-83 (P1) (Early flowering) and DC- 48 (P2) (Late flowering). Genotyping of 125 F_2 mapping population derived from the cross of DC-83 x DC- 48 using *SSR13787* SSR marker. Ladder size -50 bp DNA ladder; P1 - DC-83; P2 – DC -48 respectively.

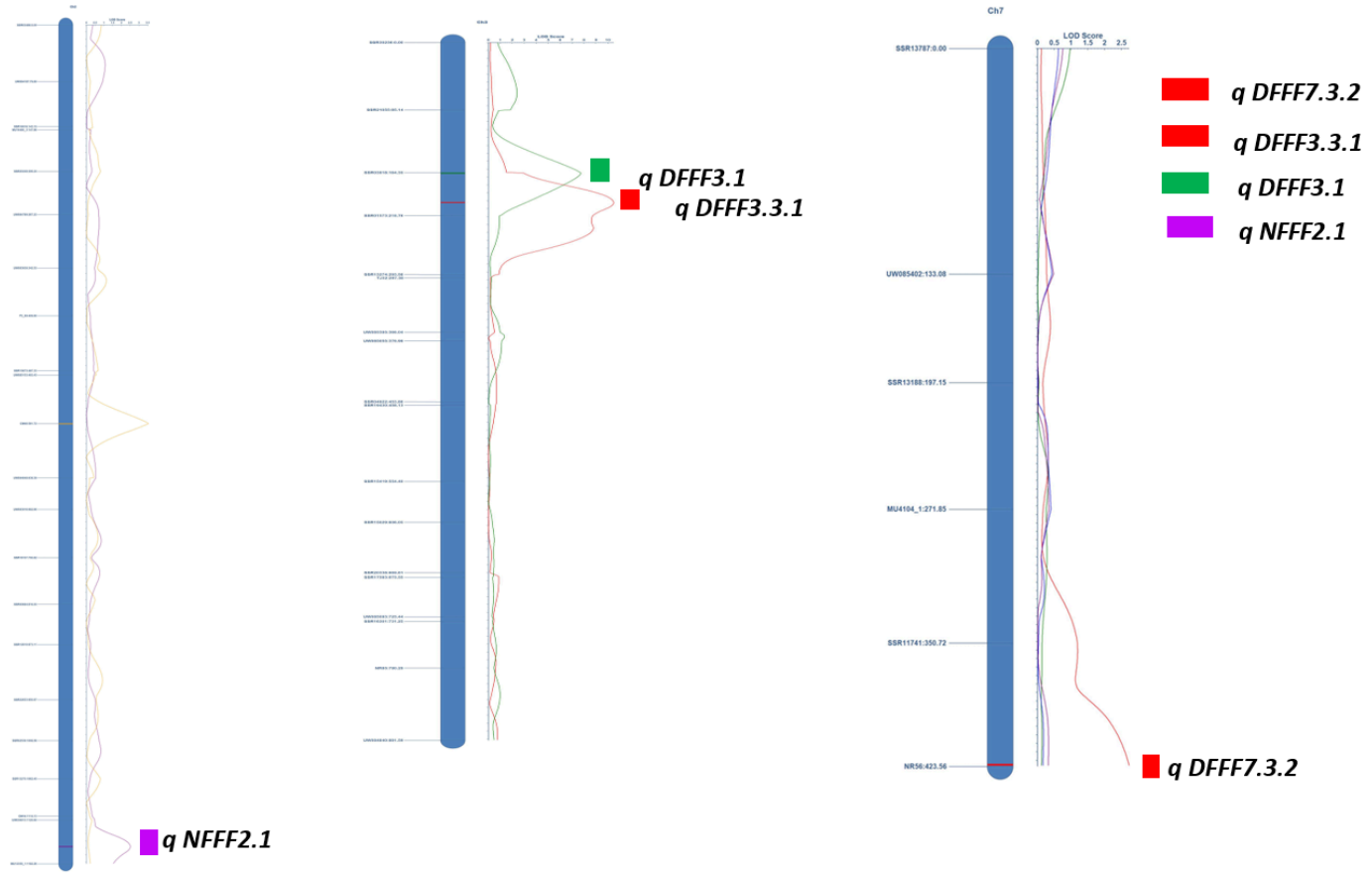

Figure 3

QTL map on Linkage groups 2 for NFFF **(A)** Linkage group 3 for DFFF **(B)** and Linkage group 7 DFFF **(C)** for flowering related traits generated in the F_2 population derived from the cross of DC-83 (P_1) (Early flowering) and DC- 48 (P_2) (Late flowering). The left side graph shows the linkage group with SSR primer position and right side of the peaks in map indicates the LOD score of respective QTLs.

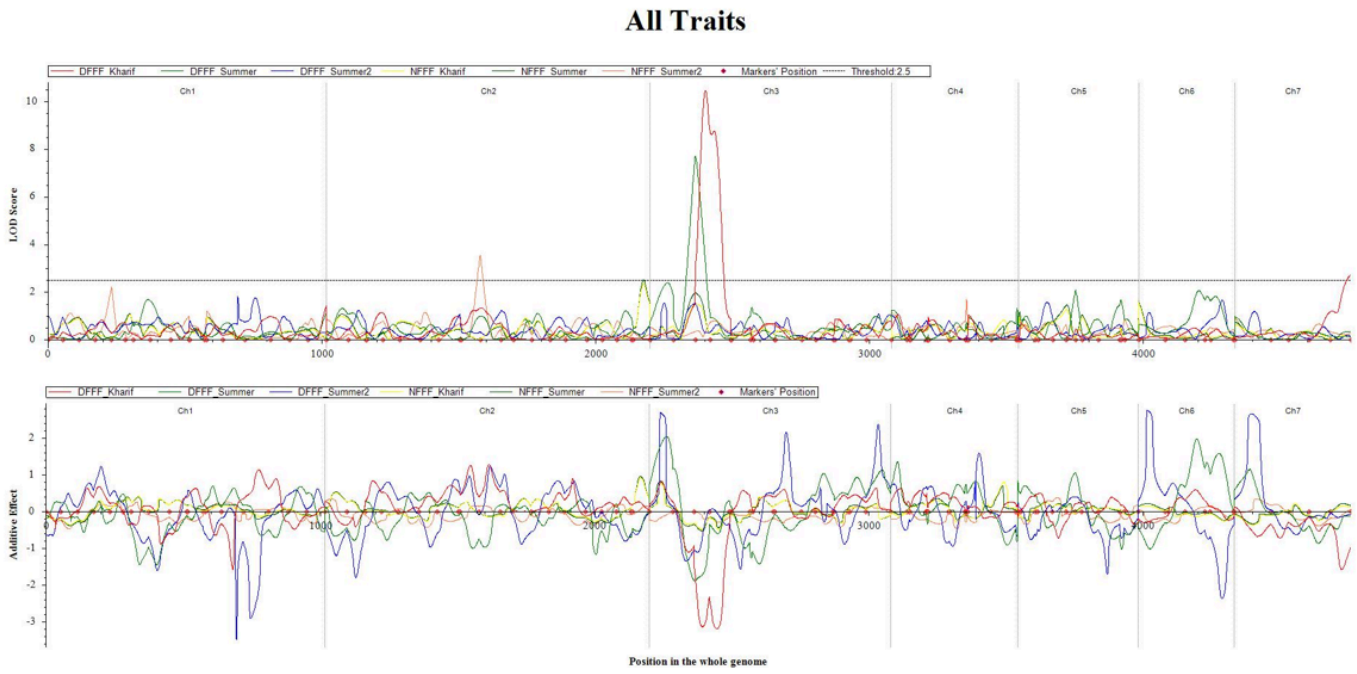

Figure 4

(A) Flowering trait; QTLs detected on all the seven-linkage group of cucumber with LOD score. **(B)** Additive effects of the detected QTLs with positions in the whole genome for flowering related traits like Days to First Female Flowering (DFFF) and Node to First Female Flowering (NFFF) in cucumber. QTL positions indicating, – ve Additive and + ve Additive values indicates the contribution from parent 2 and parent 1, respectively.

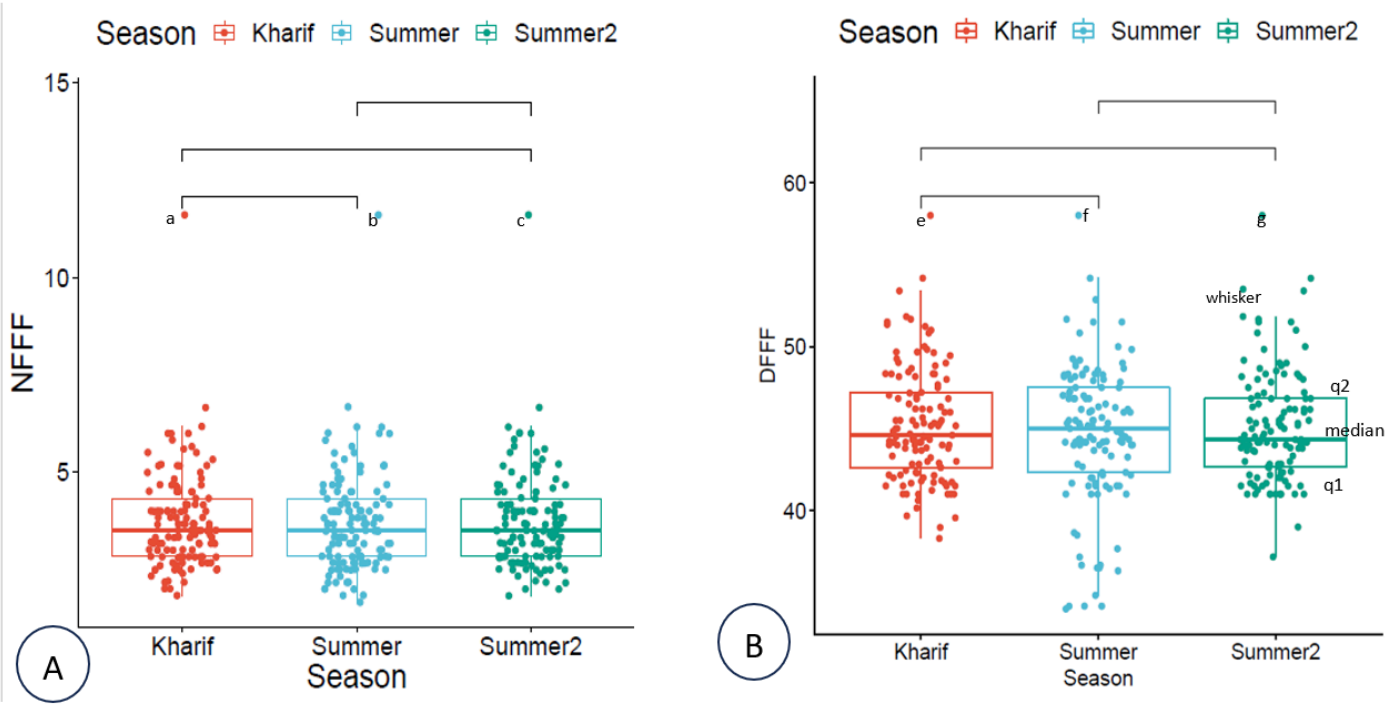

Figure 5

Box plots (A) and (B) representing the normal distribution of data points from the three-season data for flowering related traits, median (thick horizontal line in box plot) and dot points represents the data points for the traits. The dot points called outlawyers (q1+q2 = >9) which are deviated from the normal distribution are represented with letter (a,b,c,d,e,f) respectively.

Supplementary Files

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