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# Molecular tagging of maturity associated long juvenility in soybean segregants

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**Abstract**
The present study aimed to identify molecular markers associated with long juvenility and photoperiod insensitivity in soybean. $F_3$ segregants from three crosses MAUS 71 × AGS 25, MAUS 612 × AGS 25, and PP6 (EC 390977) × 291133 (EC 538828) were evaluated through both morphological and molecular analyses to validate loci responsible for these traits. Bulked segregant analysis (BSA) was employed to detect and confirm markers linked to photoperiod insensitivity and long juvenility. The long juvenile trait was successfully tagged with SSR markers *Satt197* and *Satt063* in late-sown $F_3$ segregants, providing useful tools for marker-assisted selection in soybean breeding programs.

**Keywords:** Soybean, juvenility, segregants, markers, maturity, off-season

## 1. Introduction
Soybean (*Glycine max* L. Merrill) is a short-day plant with a quantitative response to photoperiod. As a predominantly rainfed crop, it often suffers from delayed sowing due to intermittent dry spells, leading to yield loss. Early-maturing and photoperiod-insensitive varieties can improve productivity in rainfed regions and allow an additional *Rabi* crop. However, the development of very early cultivars is limited by a negative correlation between high yield and early maturity (Tardivel *et al*., 2014) [27]. Maturity duration in soybean ranges widely from less than 75 days to over 150 days, even within the same geographic region.
Photoperiod strongly influences flowering and growth habit in soybean. Late sowing or cultivation at low latitudes induces premature flowering, reducing vegetative growth and yield. Genotypes with the long juvenile (LJ) trait extend vegetative growth under short-day conditions and are therefore valuable for breeding (Cairo *et al*., 2002) [5]. However, very early cultivars are often constrained by a negative correlation between high yield and early maturity (Tardivel *et al*., 2014) [27].
Several maturity loci, designated as *E* loci, have been identified in soybean (Cober *et al*., 1996b) [8]. Eleven loci (*E1-E10* and *J*) control flowering time and maturity and have been characterized at phenotypic and genetic levels: *E1* and *E2* (Bernard, 1971) [1], *E3* (Buzzell, 1971) [3], *E4* (Buzzell and Voldeng, 1980) [4], *E5* (McBlain and Bernard, 1987) [21], *E6* (Bonato and Vello, 1999) [2], *E7* (Cober and Voldeng, 2001) [9], *E8* (Cober *et al*., 2010) [10], *E9* (Sun *et al*., 2011 [26]; Kong *et al*., 2014) [18], *E10* (Samanfar *et al*., 2017) [25], and *J* (Ray *et al*., 1995) [23]. All except *E6* and *J* delay flowering, while *E1*, *E3*, *E4*, *E7*, and *E8* are associated with photoperiod sensitivity, particularly under artificially induced long days (Cao *et al*., 2016) [6]. Among these, *E1*, *E3*, and *E4* have been specifically linked to photoperiod sensitivity under varying light conditions (Saidon *et al*., 1989) [24]. The *E3* gene has been associated with phytochrome photoreceptor genes, and a linkage between *E3* and *Dt1*, which controls determinate/ indeterminate growth habit, has also been reported (Cober and Voldeng, 1996a) [7].
Flowering and maturity in soybean are regulated by major genes whose effects vary with photoperiod. Long-day (LD) conditions enhance, while short-day (SD) conditions reduce, the influence of these loci (Xia *et al*., 2012) [31]. The *E3* and *E4* genes encode the phytochromes *GmPhyA3* and *GmPhyA2*, respectively, while two *FT* homologs, *GmFT2A* and *GmFT5A*, co-regulate flowering. The maturity genes *E1*, *E2*, *E3*, and *E4* delay flowering under LD conditions by down regulating *GmFT2A* and *GmFT5A* (Jiang *et al*., 2013) [16].

The *E3* and *E4* loci regulate flowering under specific long-day light qualities: *E3* under high red:far-red (R:FR) conditions and *E4* under low R:FR. Recessive *e3* or *e4* alleles enable flowering under their respective conditions, and both loci have been identified as Phytochrome *A3* (*E3*) and Phytochrome *A2* genes (*E4*) (Gupta *et al*., 2017) [14].

Soybean is a short-day plant, flowering when day length falls below a critical genotype-specific threshold (Destro *et al*., 2001) [12]. Sensitivity to photoperiod restricts adaptation across latitudes and limits sowing flexibility. Most maturity genes delay flowering, whereas their recessive mutant alleles promote earliness and broader adaptability. Molecular markers enable identification and transfer of such alleles into elite cultivars. From an agronomic perspective, precise control of flowering and photoperiod response is essential for maximizing yield and biomass within shorter crop durations (Cockram *et al.*, 2007) [11]. At the molecular level, allelic combinations of maturity genes, particularly *e1-e4*, influence flowering time, with *E1* exerting the strongest effect (Langewische *et al*., 2017) [19]. Beyond flowering, maturity loci also regulate photoperiodism and long juvenility.

## 2. Material and Methods

### Materials

For the present study $F_3$ seeds of three soybean (*Glycine max* (L.) Merr.) crosses were obtained from the Soybean Breeder, Agricultural Research Station, K. Digraj, District Sangli, and sown during the *Rabi* off-season (4 October 2019) at the Post Graduate Institute Farm, Mahatma Phule Krishi Vidyapeeth, Rahuri, District Ahilyanagar. The genotype AGS 25 was used as the donor parent for the long juvenile trait, while EC 390977 was the donor parent for photoperiod insensitivity. Individually tagged $F_3$ plants were phenotyped, and 20 contrasting plants from each cross were selected for molecular analysis (Table 1).

**Table 1:** Generations used

| Generations | Cross | | |
|---|---|---|---|
| | **MAUS 71×AGS 25** | **MAUS 612× AGS 25** | **EC 390977× EC 538828** |
| $P_1$ | MAUS 71 | MAUS 612 | EC390977 |
| $P_2$ | AGS 25 | AGS 25 | EC 538828 |
| $F_3$ | From selfed seed of $F_2$ | From selfed seed of $F_2$ | From selfed seed of $F_2$ |

**Phenotypic observations were taken on:**

- Plant height (cm)
- Number of pods per plant (No.)
- Days to maturity (No.)
- Test weight (gm)
- Yield per plant (gm)

### Isolation of genomic DNA

Genomic DNA was extracted from tender leaves using the modified CTAB method (Doyle and Doyle, 1987) [13]. DNA pellets were dissolved in 100 µL TE elution buffer and their concentration measured by UV visible spectrophotometer (Nanodrop, USA) at 260 and 280 nm and by 0.8% (w/v) agarose gel electrophoresis along with known amount of λ phage DNA. Based on this, all DNA samples were diluted to 20 ng $\mu L^{-1}$ with TE buffer.

### Bulking of genomic DNA of early and late maturing plants

Two contrasting DNA bulks were prepared from early and late maturing $F_3$ plants after waiting for maturity data. Each bulk comprised equimolar DNA (30 ng) from 10 plants per group across the three crosses, uniform for maturity but variable for other traits. These bulks, along with parental DNA, were used for Bulked Segregant Analysis (BSA). Markers associated with maturity were subsequently validated on all individual $F_3$ samples.

### Allele specific and SSR markers used for analysis of maturity loci in soybean cultivars

SSR markers linked to the *E6* maturity gene (Tasma *et al*., 2018) [28] were used to investigate the long juvenile trait in soybean. Additionally, allele-specific markers derived from *E3* and *E4* loci (Tsubokura *et al*., 2013) [29] were employed to differentiate the three crosses. All primers used are listed in Table 2. BSA and subsequent validation of individual $F_3$ plants were performed with these primers, with samples arranged by maturity from early too late. All primers were custom synthesized by BioServe Biotechnologies (India) Pvt. Ltd. in lyophilized form and diluted prior to use following standard procedures to ensure uniform concentration.

**Table 2:** List of Primers used in the present study

| Sr. No | Name | Sequence | Reference |
|---|---|---|---|
| 1 | *LJ Satt-197* | **F:** CACTGCTTTTTCCCCTCTCT | Tasma *et al*., 2018 [28] |
| | | **R:** AAGATACCCCCAACATTATTTGTAA | |
| 2 | *LJ Satt-063* | **F:** AAATGATTAACAATGTTTATGAT | Tasma *et al*., 2018[ 28] |
| | | **R:** ACTTGCATCAGTTAATAACAA | |
| 3 | *E4* | **F:** AGACGTAGTGCTAGGGCTAT | Tsubokura *et al*., 2013 [29] |
| | | **R:** GCATCTCGCATCACCAGATCA | |
| 4 | *e4* | **F:** AGACGTAGTGCTAGGGCTAT | Tsubokura *et al*., 2013 [29] |
| | | **R:** GCTCATCCCTTCGAATTCAG | |
| 5 | *e3-tr* | **F:** TGGAGGGTATTGGATGATGC | Tsubokura *et al*., 2013 [29] |
| | | **R:** GTCCTATACAATTCTTTACGACG | |
| 6 | *e3-Ha* | **F:**TGGAGGGTATTGGATGATGC | Tsubokura *et al*., 2013 [29] |
| | | **R:**CGGTCAAGAGCCAACATGAG | |
| 7 | *e3-Mi* | **F:**TGGAGGGTATTGGATGATGC | Tsubokura *et al*., 2013 [29] |
| | | **R:**CTAAGTCCGCCTCTGGTTTCAG | |
| 8 | *e1-as* | **F:** TCAGATGAAAGGGAGCAGTGTCAAAAGAAGT | Tsubokura *et al*., 2013 [29] |
| | | **R:**TCCGATCTCATCACCTTTCC | |

**DNA amplification Allele specific and SSR primers**

PCR amplification for maturity loci was performed in 20 µL reactions containing 60 ng genomic DNA, 1 unit *Taq* DNA polymerase, 1X PCR buffer, 2 mM $MgCl_2$, 0.25 mM dNTPs, and 15 pmol of each primer. Mastermix was prepared, dispensed into PCR tubes with DNA, and amplified in a thermal cycler with initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 1 min, 52-58 °C for 1 min (annealing), and 72 °C for 2 min, with a final extension at 72 °C for 5 min. Amplified products were stored at 4 °C until electrophoresis and visualized using a gel documentation system after electrophoresis.

## 3. Results and Discussion

The study aimed to field evaluate segregating plants from three crosses involving donor parents for long juvenility / photoperiod insentivity trait and to further tag markers linked to long juvenility (using primers that differentially amplify for LJ trait *E6* gene linked markers) and photoperiodism in soybean (using *E3* and *E4* loci specific primers).

**Morphological characters**

The morphological data of $F_3$ plants from three crosses are presented in Table 3, 4 and 5. In the cross MAUS 71 × AGS 25, the mean 50% flowering occurred 38 days after sowing. Plant height averaged 40.65 cm, ranging from 23 cm to 50 cm. Number of pods per plant varied from 5 to 62, and days to maturity ranged from 89 (#13) to 103 days (#4, #5 & #7), with maturity duration averaging 98.35 days. Test weight ranged from 7.92 (#8) to 19.60 g (#6), and yield per plant ranged 1.96 (#6)-11.90 g (#20) (Table 3).

**Table 3:** Morphological data of cross MAUS 71 × AGS 25

| Plant Number | Plant Height (cm) | No of pods | Days to Maturity | Test Weight (g) | Yield perplant(g) |
|---|---|---|---|---|---|
| MAUS 71 | - | - | 95 | - | - |
| AGS 25 | 61 | - | 115 | - | - |
| 1 | 35.5 | 53 | 98 | 10.83 | 10.29 |
| 2 | 28 | 26 | 96 | 11.76 | 5.88 |
| 3 | 41 | 40 | 99 | 9.34 | 7.57 |
| 4 | 34 | 29 | 103 | 10.88 | 4.90 |
| 5 | 40 | 59 | 103 | 8.67 | 10.41 |
| 6 | 23 | 05 | 102 | 19.60 | 1.96 |
| 7 | 45 | 34 | 103 | 9.22 | 5.26 |
| 8 | 42 | 26 | 101 | 7.92 | 7.05 |
| 9 | 40 | 50 | 94 | 11.09 | 7.21 |
| 10 | 41 | 52 | 95 | 9.32 | 6.90 |
| 11 | 39 | 21 | 95 | 9.24 | 3.79 |
| 12 | 41 | 40 | 100 | 8.82 | 7.15 |
| 13 | 40.5 | 20 | 89 | 10.54 | 4.43 |
| 14 | 43 | 26 | 97 | 9.70 | 5.63 |
| 15 | 49 | 36 | 99 | 8.82 | 6.88 |
| 16 | 50 | 59 | 98 | 8.77 | 10.44 |
| 17 | 48 | 62 | 99 | 8.47 | 10.93 |
| 18 | 40 | 34 | 98 | 9.60 | 6.34 |
| 19 | 50 | 39 | 100 | 10.76 | 7.00 |
| 20 | 43 | 59 | 98 | 9.59 | 11.90 |
| Average | 40.65 | 38.5 | 98.35 | 10.14 | 7.09 |

**Table 4:** Morphological data of cross MAUS 612 × AGS 25

| Plant Number | Plant Height (cm) | No of pods | Days to Maturity | Test Weight (g) | Yield per plant(g) |
|---|---|---|---|---|---|
| MAUS 612 | - | - | 105 | - | - |
| AGS 25 | 61 | - | 115 | - | - |
| 1 | 32 | 30 | 103 | 9.75 | 6.83 |
| 2 | 40 | 48 | 99 | 9.94 | 10.54 |
| 3 | 35 | 49 | 93 | 9.41 | 8.38 |
| 4 | 43 | 65 | 98 | 8.27 | 11.83 |
| 5 | 40 | 50 | 95 | 7.91 | 8.23 |
| 6 | 44 | 40 | 96 | 10.71 | 7.82 |
| 7 | 45 | 41 | 96 | 8.27 | 6.87 |
| 8 | 32 | 28 | 99 | 9.19 | 5.15 |
| 9 | 40 | 14 | 102 | 10.25 | 3.28 |
| 10 | 38 | 31 | 94 | 10.37 | 6.43 |
| 11 | 49 | 75 | 92 | 8.83 | 12.64 |
| 12 | 54 | 43 | 101 | 9.60 | 9.22 |
| 13 | 35 | 15 | 86 | 12.54 | 3.01 |
| 14 | 42 | 55 | 97 | 9.83 | 9.74 |
| 15 | 41 | 47 | 97 | 9.02 | 7.76 |
| 16 | 42 | 45 | 99 | 10.31 | 7.53 |
| 17 | 36 | 31 | 100 | 8.73 | 5.59 |
| 18 | 43 | 31 | 102 | 8.56 | 6.51 |
| 19 | 40 | 35 | 103 | 9.52 | 6.95 |
| 20 | 43 | 65 | 98 | 9.60 | 13.55 |
| Average | 40.7 | 41.9 | 97.5 | 9.53 | 7.89 |

In $F_3$ plants of the cross MAUS 612 × AGS 25, the mean 50% flowering occurred 36 days after sowing. Plant height averaged 40.7 cm, ranging from 32 cm to 54 cm. Number of pods per plant varied from 14 to 65, and days to maturity ranged from 86 (#13) to 103 days (#19, #1), with average maturity duration being 97.5 days. Test weight among this cross ranged from 7.91 to 12.54 g and yield per plant ranged from 3.01 to 13.55 g (Table 4).

In $F_3$ plants of the cross PP6 (EC 390977) × 291133 (EC 538828), the mean 50% flowering occurred 39 days after sowing. Plant height averaged 33.75 cm, ranging from 23 cm to 45 cm. Number of pods per plant varied from 9 to 34, and days to maturity ranged from 90 (#10) to 102 (#3, #7), with average maturity duration being 96.75 days. Test weight ranged from 10.84 (#3) to 19.71 g (#17), and yield per plant ranged 2.33 to 9.92 g (Table 5).

**Table 5:** Morphological data of cross EC 390977 × EC 538828

| Plant Number | Plant Height (cm) | No of pods | Days to Maturity | Test Weight (g) | Yield per plant(g) |
|---|---|---|---|---|---|
| EC 538828 | - | - | 87 | - | - |
| EC 390977 | 50 | - | 110 | - | - |
| 1 | 33 | 17 | 95 | 14.86 | 4.31 |
| 2 | 34 | 33 | 99 | 17.73 | 7.98 |
| 3 | 36 | 34 | 102 | 10.84 | 7.37 |
| 4 | 41 | 31 | 101 | 15.02 | 7.51 |
| 5 | 33 | 20 | 98 | 14.71 | 5.15 |
| 6 | 42 | 31 | 101 | 13.77 | 9.92 |
| 7 | 39 | 23 | 102 | 13.79 | 6.07 |
| 8 | 24 | 10 | 91 | 15.53 | 2.33 |
| 9 | 30 | 22 | 100 | 13.61 | 5.99 |
| 10 | 29 | 09 | 90 | 16.0 | 3.20 |
| 11 | 43 | 30 | 98 | 12.63 | 8.34 |
| 12 | 45 | 25 | 92 | 14.04 | 6.88 |
| 13 | 34 | 24 | 92 | 12.47 | 6.36 |
| 14 | 24 | 11 | 96 | 15.90 | 3.34 |
| 15 | 32 | 26 | 95 | 13.96 | 7.54 |
| 16 | 26 | 13 | 97 | 15.22 | 3.35 |
| 17 | 25 | 10 | 97 | 19.71 | 2.76 |
| 18 | 23 | 15 | 97 | 19.25 | 4.62 |
| 19 | 45 | 31 | 98 | 12.58 | 8.81 |
| 20 | 37 | 17 | 94 | 16.79 | 5.71 |
| Average | 33.75 | 21.6 | 96.75 | 14.92 | 5.87 |

**Analysis of LJ trait *E6* maturity loci by using SSR markers**

The *E6* gene controlling the LJ character has been mapped to chromosome 4 of soybean (Li *et al*., 2017 [20]; Yue *et al*., 2017) [32]. Its molecular identification and functional characterization provide insights into the genetic basis of the LJ trait (Li *et al*., 2017) [20]. In this study, the SSR markers LJ *Satt 197* and LJ *Satt 063*, linked to *E6* gene were used. *E6* is an allele of *J* gene which confers earliness by acting as a suppressor of functional photoperiodic *E1* gene that encodes a transcription factor that regulates photoperiodic flowering (Gupta *et al*., 2021) [15].

**PCR amplification of long juvenile trait linked SSR markers**

***LJ Satt 197***

The SSR primer *Satt 197* was used for bulked segregant and individual plant validation of the LJ trait in soybean genomic DNA from two crosses (MAUS 71 × AGS 25 and MAUS 612 × AGS 25). In bulked segregant analysis, the normal (non-LJ) parents (MAUS 71 and MAUS 612) amplified at 196 bp, while the long juvenile parent (AGS 25) amplified at 179 bp; all bands were homozygous, with occasional faint non-specific bands. Netawane (2019) [22] similarly reported a 179 bp band in long juvenile genotypes AGS 25 and DT 21, associated with long juvenile and photoperiod-insensitive types, and suggested use of *Satt* $197_{179bp}$ in marker-assisted selection (MAS).

The individual plant analysis of *Satt197* showed a consistent association with days to maturity in crosses MAUS 71 × AGS 25, amplifying fragments of either 179 bp to196 bp. Plants carrying the *Satt197*-196 bp allele, derived from the early parent MAUS 71 (DTM 95), matured earlier, between 89-99 days (7 out of 8 plants), closely aligning with the earliness of MAUS 71. In contrast, plants carrying the *Satt197*-179 bp allele, inherited from the long juvenile parent AGS 25 (DTM 115), tended to mature later, in the range of 100-103 days (4 out of 6 plants), reflecting the delayed genetic background of AGS 25. Heterozygotes exhibited maturity within 95-99 days (3 plants), lying between the two parents and suggesting partial dominance or additive interaction of the alleles. Whereas three plants did not show amplification. However, a few exceptions to this trend were noted, such as #5 (DTM 103) with *Satt197*-196 bp allele showing delayed maturity; whereas #2 (DTM 96) and #16 (DTM 98) displayed relatively early maturity although having *Satt197*-179 bp allele. Overall, *Satt197* effectively discriminated between early and late maturing segregants, with *Satt197*-196 bp allele linked to earliness and *Satt197*-179 bp allele linked to lateness, while heterozygotes displayed values intermediate to the parents. In the cross MAUS 612 × AGS 25, the $F_3$ progenies showed a wider maturity range from 86 to 103 days, irrespective of the *Satt197* allele class, reflecting variable segregation without a clear allelic trend, as no significant difference was observed in maturity period between the parents MAUS 612 (105 days) and AGS 25 (115 days) to distinguish. Thus, *Satt 197* reliably amplified an *E6* locus-specific marker (Tasma *et al*., 2018) [28]. The extensive LJ character soybean breeding in Brazil resulting in development of short-day length adapted soybean cultivars that cover 23.7% of the total world production (Gupta *et al*., 2021 [15]; Junior *et al*., 2024) [17].

***LJ satt 063***

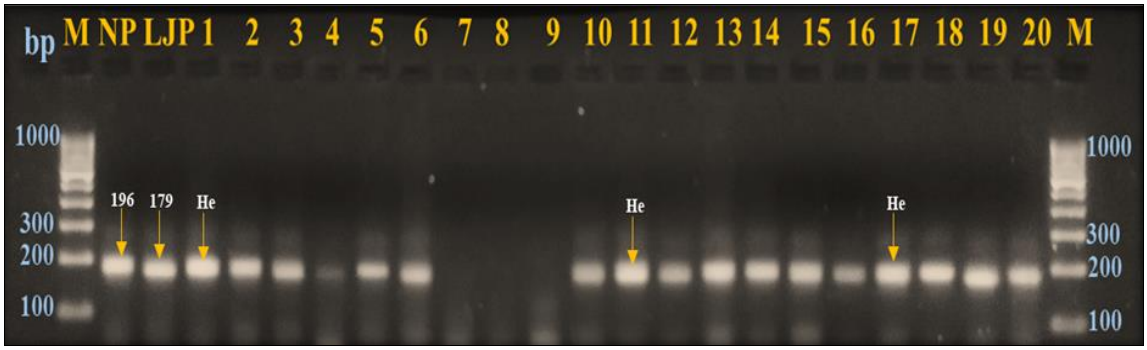
In this study, the SSR primer Satt *063* was used for bulked segregant and individual plant validation of the LJ trait in two crosses (MAUS 71 × AGS 25 and MAUS 612 × AGS 25). In bulked segregant analysis, non-LJ parents (MAUS 71 and MAUS 612) it amplified a 122 bp band, while the LJ parent (AGS 25) amplified a 116 bp band. Early and late-maturing

bulks were heterozygous, carrying both bands. Netawane (2019) [22] similarly reported a 116 bp band in LJ genotypes AGS 25 and DT 21 and highlighted its utility for MAS.

In cross MAUS 71 × AGS 25, plants carrying the *Satt063*-122 bp allele (4 out of 6) matured in the 95-98 day range, which is closely aligning with MAUS 71 parent (95 days), while plants with the *Satt063*-116 bp allele (6 out of 7) displayed delayed maturity within range of 99-103 days leaning towards the long juvenile AGS 25 parent (115 days). Heterozygotes (5 plants) expressed early to intermediate days to maturity (89-98 days), representing a broader range. Whereas two plants did not show amplification. Exceptions were notable, such as #9 with *Satt063*-116 bp allele matured earlier (94 days); while, plants #19 (100 days) and #5 (103 days) matured late even though having *Satt063*-122 bp allele which did not conform to the general tendency. Overall, the allelic groups overlapped considerably and heterozygotes exhibited wider variation. Similarly in cross MAUS 612 × AGS 25, plants carrying the *Satt063*-122 bp allele (2 out of 2) matured between 96-98 days aligning little close with the parent MAUS 612 (105 days), while those with the *Satt063*-116 bp allele (3 out of 4) exhibited range of 99-103 days, having relatively delayed maturity as like long juvenile parent AGS 25 (115 days). However the plant #5 marked exception of having early maturity (95 days) though having *Satt063*-116 bp allele. The heterozygotes (14 plants) formed the wide range of 86-102 days, thus spanning the entire segregating range. In individual DNA validation, this primer amplified an *E6* locus-specific marker as earlier reported by Tasma *et al*. (2018) [28]. They suggested that breeding for LJ characters can help to improve soybean productivity in low latitude tropical countries like Indonesia. Recently used SSR markers have been used for development of soybean adopted for short photoperiod tropical areas (Wibisono *et al*., 2025) [30].

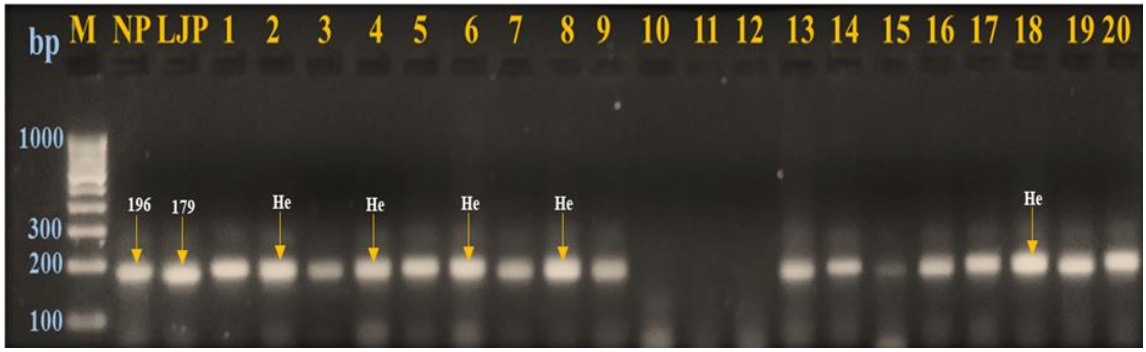
**PCR amplification and analysis of *E1*, *E3* and *E4* locus specific markers**

- ***E4* marker:** The *E4* locus-linked allele-specific CAPS marker (Tsubokura *et al*., 2013) [29] was used to score bulked DNA samples from three soybean crosses. The *E4* primer pair amplified two monomorphic bands at 58°C annealing temperature (225 bp and 325 bp) in MAUS 71 × AGS 25 and MAUS 612 × AGS 25. In MAUS 71 × AGS 25, the early bulk was homozygous (325 bp) while the late bulk was heterozygous (225 bp and 325 bp). In MAUS 612 × AGS 25, both early and late bulks were heterozygous (225 bp and 325 bp). No amplification was observed in EC 390977 × EC 538828, including the photoperiod-insensitive parent (EC 390977), despite multiple attempts. As monomorphism was observed in bulked samples, no further individual validation was performed. These results indicate the *E4* CAPS marker can detect allelic variation in flowering time in some soybean crosses but may not amplify in photoperiod-insensitive genotypes.
- ***e3-tr* marker:** The *E3* locus-linked dCAPS marker (Tsubokura *et al*., 2013) [29] was used to score genomic DNA from three soybean crosses, the e3-tr primer pair amplified 300 bp and 900 bp bands at 58°C annealing temperature in MAUS 71 × AGS 25 and MAUS 612 × AGS 25, with heterozygosity except for two homozygous plants (#3 and #20) in MAUS 612 × AGS 25. In EC 390977 × EC 538828, only a 300 bp band was observed. Amplified sizes differed slightly from the expected 274 bp (without restriction digestion by restriction enzyme *MseI*) and 324 bp (after MseI digestion; Tsubokura *et al*., 2013) [29]. EC 390977 parent is reported as *E1E1 E2E2* e3-*tr e3-tr E4E4* (Gupta *et al*., 2017) [14]. Plants with -e3 genotype identified via FLP were either *E3e3* or *e3e3* by SSR. MseI digestion could not be performed due to unavailability of the enzyme.
- ***e3-Ha* marker:** The *E3* locus-linked dCAPS marker (Tsubokura *et al*., 2013) [29] was used to score genomic DNA from three soybean crosses. On 1.2% agarose gel, the *e3-Ha* primer pair amplified 2 monomorphic 300 bp and 600 bp bands at 58 °C C annealing temperature. In MAUS 71 × AGS 25, 1 early plant (#6) and 5 late plants (#14, #17 #18 #19 #20) were heterozygous. In MAUS 612 × AGS 25, 5 early and 8 late plants were heterozygous with band size 300 bp and 600 bp. In EC 390977 × EC 538828, all 10 early and 10 late plants were heterozygous. Observed band sizes differed slightly from the expected 558 bp (Tsubokura *et al*., 2013) [29].
- ***E3-Mi* marker:** The *E3* locus-linked dCAPS marker (Tsubokura *et al*., 2013) [29] was used to score genomic DNA from three soybean crosses. The *e3-Mi* primer pair showed no amplification in MAUS 71 × AGS 25 and MAUS 612 × AGS 25. In EC 390977 × EC 538828, a 250 bp homozygous band was observed at in all early and late plants except 1 early plant (#3), where amplification was absent. The observed size differed from the expected 1339 bp (Tsubokura *et al*., 2013) [29].
- ***E1-as* marker:** The *E1* locus-linked allele-specific CAPS marker (Tsubokura *et al*., 2013) [29] was used to score genomic DNA from three soybean crosses. The *e1-as* primer pair amplified 2 monomorphic 300 bp and 600 bp bands without restriction digestion. In MAUS 71 × AGS 25, 1 early plant (#6) and 5 late plants (#14, #17, #18, #19 and #20) were heterozygous. In MAUS 612 × AGS 25, 5 early and 7 late plants were heterozygous. In EC 390977 × EC 538828, all 10 early and 10 late plants were heterozygous. The observed band sizes differed from the expected 412 bp after *Taqα*-I digestion (Tsubokura *et al*., 2013) [29].



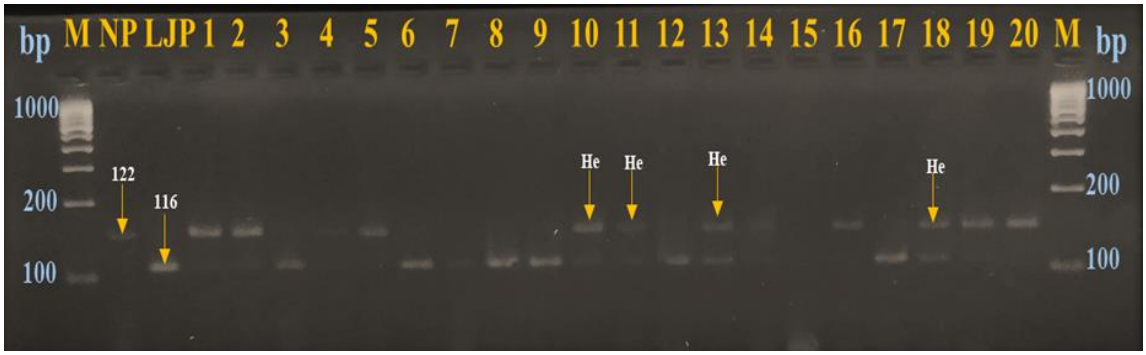

M-100 bp ladder, NP-Normal Parent, LJP-Long Juvenile Parent, He-Heterozygous.

**Plate 1:** Individual sample PCR amplification profile of *satt 197* of Cross I-MAUS 71 × AGS 25



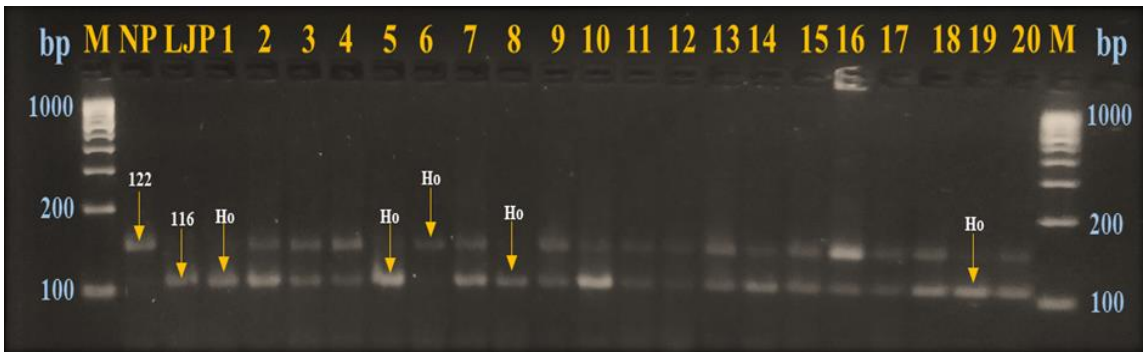

M-100 bp ladder, NP-Normal Parent, LJP-Long Juvenile Parent, He-Heterozygous

**Plate 2:** Individual sample PCR amplification profile of *satt 197* of Cross II-MAUS 612 × AGS 25



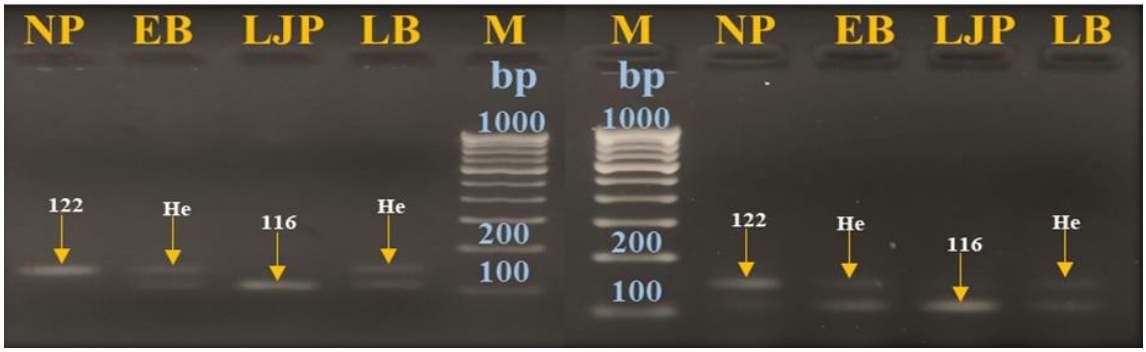

M-100 bp ladder, NP-Normal Parent, LJP-Long Juvenile Parent, He-Heterozygous

**Plate 3:** Individual sample PCR amplification profile of *satt 063* of Cross I-MAUS 71 × AGS 25




M-100 bp ladder, NP-Normal Parent, LJP-Long Juvenile Parent, He-Heterozygous, Ho-Homozygous

**Plate 4:** Individual sample PCR amplification profile of *satt 063* of Cross II-MAUS 612 × AGS 25




M-100 bp ladder, NP-Normal Parent, LJP-Long Juvenile Parent, EB-Early Bulk, LB-Late Bulk, He-Heterozygous

**Plate 5:** PCR amplification profile of *satt 063* on Bulk Segregant Analysis of Cross I-MAUS 71 × AGS 25 and Cross II-MAUS 612 × AGS 25

## 4. Conclusion

The present study demonstrated the utility of SSR markers *Satt197* and *Satt063* in characterizing allelic variation at the E6 locus associated with the long juvenile (LJ) trait in soybean. *Satt197* showed a clear association with days to maturity, effectively grouping segregants into early, late, and intermediate classes, thereby reflecting the genetic contribution of parental alleles. Similarly, *Satt063* successfully distinguished allele classes, where homozygotes formed relatively consistent groups, while heterozygotes displayed wider variability, contributing to greater segregation in maturity duration. Overall, both markers proved reliable for identifying allelic differences at the *E6* locus

and offer valuable tools for marker-assisted selection in soybean breeding for maturity regulation and LJ trait improvement.

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