

Full Length Research Paper

Assessment of molecular markers associated with photosensitivity in cowpea [*Vigna unguiculata* (L.) Walp.] recombinant inbred lines

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The length of daylight is an environmental factor that affects the productivity of many plant species, such as some cowpea varieties that only flower during periods of short days. This work aimed to identify the putative molecular markers associated with the sensitivity to day-length variation in cowpea. This study was conducted on the varieties Bambey 21 (non-photosensitive) and Ndout (photosensitive short-day), as well as their 77 recombinant line (RIL) from the 9th generation. These plants were grown during the long days to determine their photosensitivity. Bulk segregant analysis (BSA) was used on these genotypes, with 105 simple sequence repeat (SSR) primers used to discriminate between the parents. Subsequently, 22 cowpea cultivars with different photosensitivities were screened for putative markers. Amplified deoxyribonucleic acid (DNA) fragments associated with photosensitivity were sequenced and aligned with those of the non-photosensitive cultivar and the National Center for Biotechnology Information (NCBI) database. Among the 10 SSR markers related to photosensitivity according to the BSA, only SSR-6258 and SSR-6323 were specific for the Ndout variety and RIL that were photosensitive. SSR-6258 produced a band (330 bp) located on chromosome 4 only in photosensitive varieties. This sequence is 72% identical to the non-photosensitive one, with 20 SNPs (single-nucleotide polymorphisms). This DNA fragment has 86.14% similarity with CLV2, which is involved in the flowering process. As for the SSR-6323 marker, its sequence is identical to a portion of the 28-like ubiquitin-conjugated enzyme E2 gene of *V. unguiculata*, involved in collaboration with the COP1 gene encoding the E3 enzyme, in the control of photoperiodic flowering. These markers identified in this study could help cowpea breeders in marker-assisted selection (MAS) from the seedling stage to determine photosensitive genotypes.

Key words: Photosensitivity, *Vigna unguiculata*, bulked segregant analysis, simple sequence repeat.

INTRODUCTION

Cowpea is a highly appreciated legume for its leaves, which constitute a real protein resource, containing immature pods, and, in particular, for its mature seeds, between 23 and 32% protein (Nielsen et al., 1993). Its

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low cost makes it a food that can fill protein deficits for poor people in developing countries. Cowpea can play an important role in the diets of developed countries, as it has a low lipid content (1.4 to 2.7%) and 6% dietary fiber (Bressani, 1985; Nielsen et al., 1993). Its seeds are also rich in minerals (iron, calcium, zinc, etc.) and vitamins, including B vitamins such as vitamin B9 (folic acid) (Hall et al., 2003). In addition, its leaves provide important fodder for livestock in the Sahelian zone and the Great Lakes region (Tarawali et al. 1997).

Economically, the sale of mature seeds, unripe pods, and hay stored during the dry season is an important source of additional income for rural communities. Cowpea can grow on many types of soil, due to its ability to establish an atmospheric nitrogen (N₂) fixing symbiosis with various bacterial strains belonging to the genus *Bradyrhizobium* (Suh and Simbi, 1983) and to enter into symbiosis with microscopic soil fungi (*endomycorrhizae*), thus increasing its uptake of water and mineral substances, particularly phosphorus (Gueye, 1983). Hence, its cultivation on some 12.5 million ha, including 9.8 million in West Africa, with annual world production of dry seeds varying between 3.1 and 3.3 million tons (Food and Agriculture Organization of the United Nations [FAO], 2010), 70% of which is produced in Africa (Singh et al., 2002). Despite this importance, cowpea production is hampered by several constraints (biotic and abiotic), such as photosensitivity.

Indeed, for many flowering plants, the transition from the vegetative to the reproductive stage is controlled by day length, namely photoperiod. This ability is genotype-dependent in cowpea, as some varieties flower only during short day (SD) periods, called photosensitive varieties, and others flower regardless of day length, called non-photosensitive varieties (Njoku, 1958; Hadley et al., 1983; Nuhu and Mukhtar, 2013). This ability to flower according to day length is a handicap for photosensitive cowpea varieties, because since the 1960s, the Sahelian zone has been experiencing a significant reduction in the length of the rainy season (from 90 to around 60 to 70 days) and in the amount of annual rainfall. As a result, farmers are less likely to grow photosensitive varieties, such as the super-nodulating Ndout variety (Ndiaye et al., 2000), which is highly appreciated by the population for its taste but reduces the genetic diversity of Senegalese cowpea germplasm.

This mechanism of regulation of morphogenesis has not yet been elucidated in cowpea, raising the need to investigate the molecular markers associated with photosensitivity. However, Pooprompan et al. (2006) identified two QTL (quantitative trait loci) associated with the number of days to flowering trait in recombinant soybean lines (RIL), which are very close to simple sequence repeat (SSR) markers (Satt132 and Satt431). Also, Sun et al. (2019) have located a QTL linked to flowering time, on chromosome 16, which contains an orthologue of the flowering locus T, FT5a. Furthermore,

the study of genetic variation in flowering time as a function of photoperiod in common bean revealed a major locus controlling photoperiod sensitivity on chromosome 4 (González et al., 2021). In addition, the molecular regulation of flowering, focusing on the E3/E4-E1-FT regulatory module, has been identified as a central regulator of the flowering pathway in soybean (Wang et al., 2023). Recently, Wang et al. (2024) showed that the soybean E2 locus (homologous to Arabidopsis GIGANTEA), which is involved in photoperiod sensitivity, interacts with the ZEITLUPE 2 (ZTL2) gene to mediate its degradation, resulting in early flowering; however, FKF1 stabilizes E2 and delays flowering. The balance between the degradation by ZTL2 and the stabilization by FKF1 allows the soybean to regulate the flowering time and to maximize the grain yield.

In this study, BSA was used, a method developed by Michelmore et al. (1991). This method was used in cowpeas to identify molecular markers linked to *Striga gesnerioides* race 1 resistance genes (Ouédraogo et al., 2002) to detect molecular markers linked to abiotic factors. Furthermore, BSA has enabled the identification of SSR markers related to heat tolerance in *Oryza sativa* (Zhang et al., 2009), *Triticum aestivum* (Barakat et al., 2012), to cold tolerance in maize (Farooqi et al., 2016), and the duration of almond flowering (Rasouli et al., 2013). Many studies have been done on photosensitivity, but this work is the first study carried out on cowpea to best of our knowledge.

For this reason, this work aimed to identify the SSR markers associated with sensitivity to day-length variation in cowpea.

MATERIALS AND METHODS

Plant and RIL population development

Two cowpea varieties, Ndout yellow pods (YP) and Bambey 21, were crossed to develop a recombinant line (RIL). Ndout YP is SD photosensitive, while Bambey 21 is non-photosensitive. The RILs were developed until F₉ with the Single Seed Descendant (SSD) method.

Population screening for photosensitivity

The healthy seeds of both parents and 77 RILs were sown in plastic bags filled with a mixture of soil and potting soil. Each bag contained one seed and was watered 3 days a week with tap water. The seedlings were then placed in the Université Cheikh Anta Diop de Dakar (UCAD) Plant Biology Department shade house during the long day period (13 to 14 h) from April to August. This corresponds to the period of long days, when photosensitive cowpea lines do not flower, but non-photosensitive genotypes do. This allowed us to identify the photosensitive and non-photosensitive RILs.

Deoxyribonucleic acid (DNA) extraction and BSA

DNA was extracted from three young leaflets (15 days after

Table 1. List of 22 cowpea varieties used in this study, their photosensitivity, and pedigree.

Variety	Pedigree	Photosensitivity
2065	Mouride × 58-77	No photosensitive
58-153	Local	No photosensitive
58-184	Local	No photosensitive
58-191	Local	No photosensitive
58-3	Local	No photosensitive
58-53	Local	No photosensitive
58-57	Local	No photosensitive
58-74F	Local	No photosensitive
58-77	Local	No photosensitive
58-78	Local	No photosensitive
58-80	Local	No photosensitive
Bambey 21	5/8 of 58-40+1/4 of 66-74+1/8 of 58650	No photosensitive
Melakh	IS86-292 × IT83s-742-13	No photosensitive
Mougne	58-74 × Pout	No photosensitive
Mouride	58-57 × IT81D-1137	No photosensitive
Ndiaga Aw	Local	No photosensitive
Ndiambour	58-41 × 58-57	No photosensitive
Diongama	58-57 × IT81D-1137	No photosensitive
Yacine	Ndiaga Aw × B89-504	No photosensitive
Ndout violet pods	Local	Photosensitive
Ndout yellow pods	Local	Photosensitive
Baye Ngagne	Local	Photosensitive

sowing) of each plant according to the protocol described by Badiane et al. (2012), quantified using an ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and stored at 4°C. Then, the DNA samples were pooled into nine batches, each containing 50 ng/μl of DNA. Seven of the batches consisted of DNA extracted from ten distinct photosensitive plants, and the remaining two comprised DNA derived from three nonphotosensitive genotypes. The BSA requires the prior identification of SSR discriminant primers between the two parent varieties, Ndout YP and Bambey 21. Thus, 353 microsatellite primers identified in Cowpea, collected in Gene-space Sequence Reads (GSRs) and Expressed Sequence Tag (EST), were used during this experiment (Timko et al., 2008). All primers were tested on both parents to identify the most discriminant markers.

DNA amplification and electrophoresis

Polymerase chain reaction (PCR) amplification was performed in a 0.2 mL PCR tube containing a final volume of 25 μL reaction mixture. Each reaction contained 2.5 μL of 10X PCR buffer, 1 μM of each primer, 1 mM of each dNTP, 0.5 units of Taq DNA polymerase, and 50 ng of template DNA. The reactions were run in an Eppendorf Mastercycler Gradient thermocycler programmed as follows: an initial denaturation at 94°C for 1 min; 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min; followed by a final extension at 72°C for 10 min. Amplified PCR products were resolved on 6% polyacrylamide gel and 0.5 × TBE buffer containing 1 mg/mL ethidium bromide at 300 V (C-DAS-400-50 system, C.B.S. Scientific Co., Delmar, CA, USA). DNA bands were visualized under UV light using an Alphascreen 2200 (Alpha Innotech, Santa Clara, CA, USA).

Screening cowpea germplasm with a segregating marker associated with photosensitivity

To confirm or not their association with photosensitivity, PCR reactions were performed on the DNA of 22 local cowpea varieties (Table 1) using the SSR markers specific for the photosensitive genotypes based on the BSA technique.

Sequencing of DNA fragments associated with photosensitivity

Purification of amplicons

The amplified DNA fragments were visualized using UV at 320 nm, then excised with a scalpel. The DNA was isolated from agarose gel using the manufacturer's protocol [Zymoclean™ Gel DNA Recovery Kit D4001, D4002, D4007, and D4008 (www.zymoresearch.com)] with slight modifications. Finally, the DNA was washed with 200 μl of wash buffer (Zymoclean™), followed by centrifugation at 13,200 rpm for 50 s, eluted in 15 μL of TE, and stored at 5°C for 24 h. DNA samples were run on an agarose gel (0.8% in TAE buffer × 1) at 100 volts for 1 h to check the quality of the purified DNA, visualized by UV at 320 nm, and photographed using Alphascreen 2200 software.

Sequencing reaction

The sequencing reaction was carried out with 50 ng of DNA, 4 μl of Big Dye × 2.5 mix (BigDye® Terminator v3.1 Cycle Sequencing Kit), 2 μL of Big Dye × 5 buffer, and 3.2 pmol of primer SSR-6258.

For each fragment, both strands (forward and reverse strands) were sequenced in a final volume of 20 µL. Then, the tubes were loaded in a thermocycler programmed at 96°C, 1 min for denaturation, followed by 25 cycles of 10 s at 96°C for denaturation, 5 s at 50°C for hybridization, and 4 min at 60°C for elongation. At the end of the sequencing reaction, DNA was precipitated with 2 µL of 3 M sodium acetate and 50 µL of 100% ethanol. The tube was vigorously vortexed, incubated at room temperature (25°C) for 15 min, and centrifuged at 13,200 rpm for 20 min. The supernatant was removed, and the DNA was washed with 200 µL of 70% ethanol by centrifugation at 13,200 rpm for 5 min. The supernatant was removed, and the DNA was dried at room temperature for 20 min. Then, 30 µL of deionized formamide (minimum 99.5%, GC, Sigma) was added, and the tubes were vortexed and briefly centrifuged. The DNA was denatured at 92°C with the thermocycler for 3 min, cooled in ice for 5 min, transferred to "96 well plates" tubes, and loaded into the sequencer (Applied Biosystems 3130xl Genetic Analyzers). The sequence of the DNA fragment was read using FinchTV software.

Bioinformatic analysis

The DNA sequence associated with photosensitivity isolated from Ndout (YP and PP) and Baye Ngagne was aligned using the BioEdit software version 7.7. A BLAST search was performed to find their homologous sequences in the GenBank database. Eleven sequences were used to construct a phylogenetic tree, including sequences from Ndout (YP and PP) and Baye Ngagne. A phylogenetic tree was performed by MEGA12 software (Molecular Evolutionary Genetics Analysis version 12) (Tamura et al., 2021) using the Neighbor-Joining method and with 1000 bootstrap replications using the Maximum-Likelihood (ML) methods (Felsenstein, 1985).

RESULTS

Screening for photosensitivity

Using a single seed descent pattern up to the F9 generation allowed us to identify among the 77 RILs, photosensitive and non-photosensitive genotypes. Thus, 92.2% (71 genotypes) are photosensitive, while 7.8% (6 genotypes) are non-photosensitive.

Bulked segregant analysis (BSA)

Identification of putative markers linked to photosensitivity

This analysis revealed that Ndout YP and Bambey 21 were discriminated by 105 out of 353 primers, of which 10 were associated with photosensitivity (Table 2). Indeed, a specific band was amplified only in the photosensitive genotypes of the RIL and the parent Ndout YP. While it was absent in the parent Bambey 21 (non-photosensitive) and the non-photosensitive bulks (Figure 1A). In addition, primers SSR-6258, SSR-6284, SSR-6292, SSR-6304, SSR-6323, SSR-6531, and SSR-6906 produced a band specific to the photosensitive parent in one of the non-photosensitive bulks, as

illustrated by markers SSR-6258 (Figure 1B) and SSR-6323 (Figure 1C).

Verification of the relationship between SSR markers and photosensitivity

Among the 10 primers amplifying a DNA fragment associated with photosensitivity based on BSA analysis, only the SSR-6258 marker confirmed its association. It amplified a DNA fragment of 330 bp only in the photosensitive varieties Ndout yellow pods, Ndout purple pods, and Baye Ngagne, while it was absent in the 19 other non-photosensitive varieties (Figure 2).

Bioinformatics analysis

Variation of the DNA fragment amplified by SSR-6258 and prediction of its function

Alignment of the DNA fragment amplified by the SSR-6258 primer showed 100% similarity between the photosensitive genotypes (Ndout YP, Ndout PP, and Baye Ngagne), but a 72 bp deletion was observed in the non-photosensitive Bambey 21 (Figure 3). This sequence shares 72% similarity with the one detected in the photosensitive individual and shows 20 SNPs (Single Nucleotide Polymorphism).

NCBI Blasts search using the DNA fragment amplified with primer SSR-6258 in the photosensitive genotypes showed that this marker is located on chromosome 4, as its very low E-value equals 6.10^{-100} with a coverage rate of 97%, and a maximum score of 375 (Table 3). The prediction showed that it corresponds to the gene coding for receptor-like protein 12 (*RLP12*) of *Vigna angularis*, with a very low E-value equal to 2.10^{-40} and a maximum score of 178 (Table 3). It would cover the area of the gene from base 1091 to 1255 (Figure 4). In fact, this sequence is 96% covered with a similarity of 86.14%. Moreover, the alignments of the DNA sequence of the non-photosensitive variety revealed that it would be on the same chromosome 4 of *Vigna unguiculata*, with an E value equal to 6.10^{-109} , 96% coverage, 95.98% similarity, and a maximum score of 405 (Table 3). As in photosensitive varieties, this sequence would code for the *RLP12* gene of *V. angularis*, with an E value of 3.10^{-37} , 96% coverage, a similarity up to 85.62% and a maximum score of 167 (Table 3, Figure 5). Thus, a difference of 0.52% similarity is noted with the *RLP12* gene between photosensitive and non-photosensitive varieties.

On the other hand, the blast of sequences of the remaining 9 SSR markers revealed their probable assignment to specific chromosomes, but SSR-6323 showed a perfect identity with the E2 28 ubiquitin-conjugated enzyme gene of *V. unguiculata*, *Asparagus officinalis*, and *Coprinopsis cinerea* with an E value equal to 0.53, 0.93, and 0.93, respectively (Table 4).

Table 2. Primer sequences for DNA-specific fragments associated with BSA photosensitivity and the size of bands produced.

Primer code	Sequences (5'-3')	Band size (bp)
SSR-6258	F: GGTTCCTAGTTGGAAGGAA R: ATTATGCCATGGAGGGTTCA	330
SSR-6284	F: GAAAGGGAAGGATTATGGGATA R: GGCAAATAGCGGGGTAGAGT	134-154
SSR-6292	F: AAGGGTGCACTGGTAGAGGA R: GCTCACTTTGTGCATGTTCC	298
SSR-6302	F: TGGAGGCATAAAATGACACCT R: AAGCTGATTGTGGAACCATG	154-201
SSR-6304	F: CTGGAACAAGTCGAGATGGAA R: CCATCCCCACCAAAAGT	154
SSR-6314	F: TGGAGGCATAAAATGACACCT R: TGAAGCTGATTGTGGAACCAT	154-201
SSR-6323	F: CAAAGGGTCATCAGGATTGG R: TTTAAGCAGCCAAGCAGTTGT	220-298
SSR-6531	F: TGTATTTTAGAAGATGGAAG R: GGTTTTGTTCTCATTCATTC	517-1018
SSR-6876	F: GGAATTGAAATTGATCTAATG R: GTATTTAAGTGGCTTATGAGGTTG	75-134
SSR-6906	F: GGACATTTAGGATTGGGTGG R: CAAGAATGTCTGAACTAATATGC	220-298

R = Reverse primer, F= forward primer.

Relationship between photosensitivity-related DNA sequences and databases

The hierarchical ascending classification of the DNA fragments associated with photosensitivity and the homologous sequences downloaded from NCBI, highlights 3 groups of probable proteins (Figure 6). The first group consists of proteins with 71% similarity, which are mainly probable leucine-rich repeat-receptor-like protein kinase (LRR-RLK) of *Vigna radiata* var. *radiata* and receptor-like protein kinase 2 (RLK) of *V. angularis*. The second group shows a 98% similarity between the sequence of Ndout (YP and PP) and Baye Ngagne related to photosensitivity and the probable receptor-like protein 12 (RLP12) of *V. angularis*. In the same group, the sequence of Ndout (YP and PP), Baye Ngagne, and a part of *V. angularis* chromosome 11 are 96% identical. The third group includes probable receptor-like proteins (EIX1 and 12) and an inactive leucine-rich repeat receptor kinase, XIAO.

DISCUSSION

The BSA technique has been used to identify markers related to abiotic factors (Hennell et al., 2017; Huang et al., 2014; Jang et al., 2008). This study showed that 10 putative SSR markers (SSR-6876, SSR-6302, SSR-6531, SSR-6258, SSR-6284, SSR-6292, SSR-6302, SSR-6304, SSR-6314, and SSR-6323) would be linked to photosensitivity in cowpea (Table 2). And screening the 22 cowpea varieties from the Senegalese germplasm with these markers revealed that the SSR-6258 amplified a fragment of 330 bp, specific to photosensitive varieties (Figure 2).

Alignment of the sequences produced by the SSR-6258 marker in the varieties Ndout and Baye Ngagne (photosensitive), revealed that they are identical, but show 72% similarity with the one produced in Bambey 21 (non-photosensitive) with a deletion of 72 bp (Figure 3). Since cowpea is generally a short-day (SD) photosensitive plant (Ehlers and Hall, 1996), the

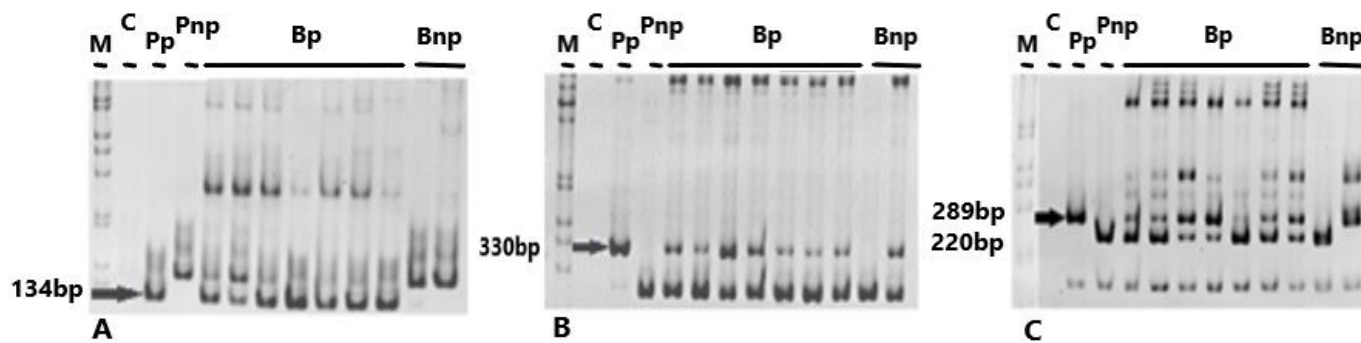


Figure 1. Bulk gels with (A) SSR-6876, (B) SSR-6258, and (C) SSR-6323. M: Marker, C: Control, P_p: Photosensitive parent (Ndout), P_{NP}: Non-photosensitive parent (Bambey 21), B_p: photosensitive bulks, and B_{NP}: non-photosensitive bulks.

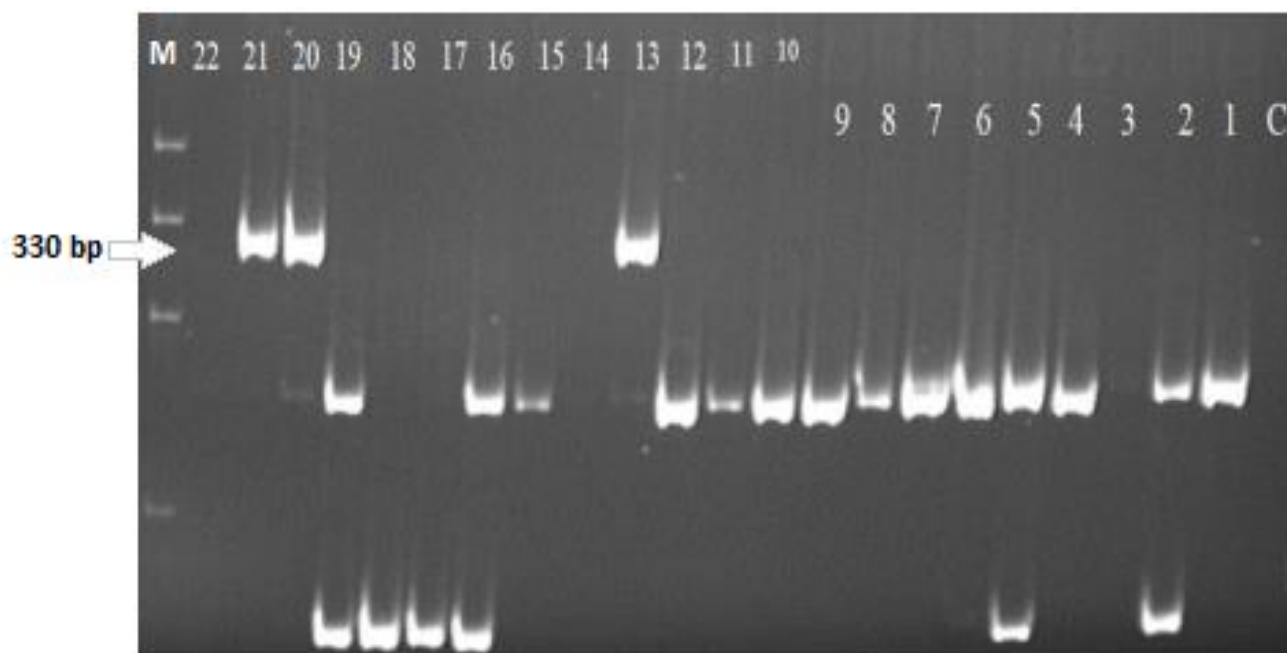


Figure 2. Electrophoretic profile of the SSR-6258 marker on the 22 cowpea varieties. 1: 53-3, 2: 58-53, 3: 58-57, 4: 58-74F, 5: 58-77, 6: 58-78, 7: 58-80, 8: 58-153, 9: 58-184, 10: 58-191, 11: 2065, 12: Bambey21, 13: Baye Ngagne, 14: Diongoma, 15: Melakh, 16: Mougne, 17: Mouride, 18: Ndiaga Aw, 19: Ndiambour, 20: Ndout yellow pods, 21: Ndout violet pods, and 22: Yacine.

presence of non-photosensitive varieties could be linked to the invalidation of one gene by deleting a functional region involved in photosensitivity during the course of evolution.

NCBI Blast search of the sequence amplified by SSR-6258 in the photosensitive varieties in the NCBI database revealed a similarity to receptor-like protein 12 (RLP12), leucine-rich repeat-receptor-like protein kinase (LRR-RLK), and leucine-rich repeat-receptor-like protein (LRR-RLP) (Tables 3 and 4 and Figures 4, 5 and 6).

The classification by ascending hierarchy revealed that the photosensitivity-linked sequence according to this

work is very close to the genes encoding LRR-RLKs (Table 3 and Figure 6). These are proteins with a predicted signal sequence, a transmembrane region, and a cytoplasmic kinase domain, which largely mediate cellular signal transduction in plants (Shiu and Bleecker 2001). They play a role in a wide range of processes in plants. LRR-RLKs are derived from several genes, including CALVATA1 (CLV1), which plays a central role in the maintenance of the shoot apical meristem (SAM) (Clark et al., 1997). Despite the fact that photosensitivity-related genes have been known for years, direct implication of CALVATA genes on photosensitivity was

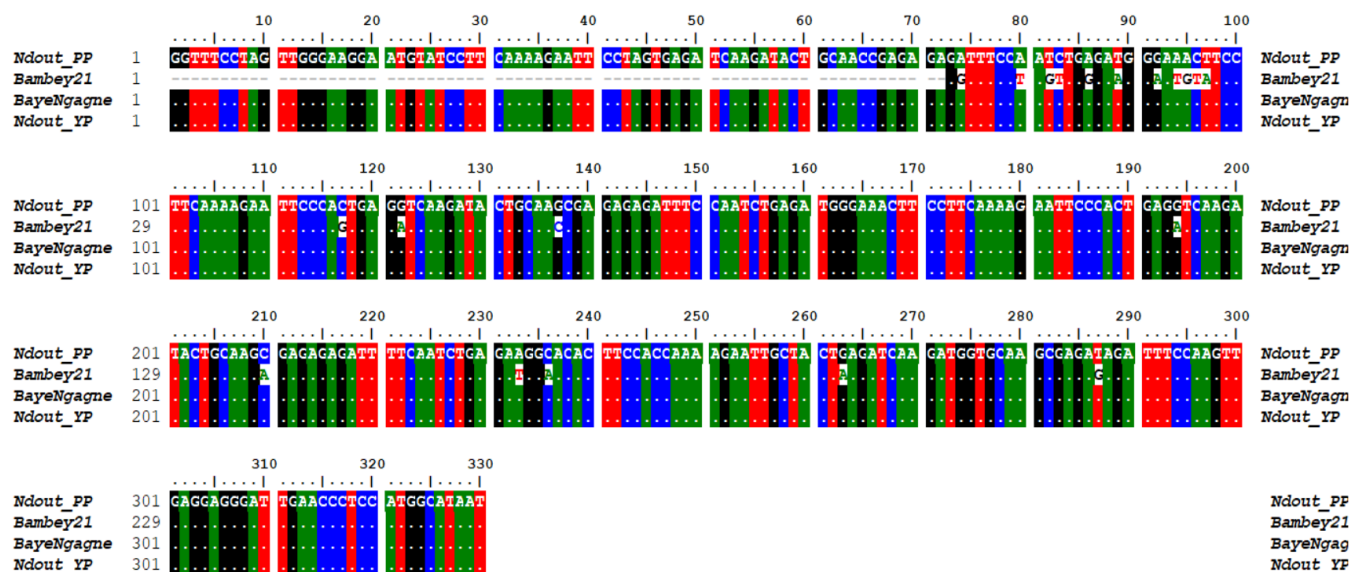


Figure 3. Alignment of DNA sequences produced by primer SSR-6258 in photosensitive varieties: Baye Ngagne, Ndout YP (yellow pod), and Ndout PP (purple pod), and non-photosensitive variety (Bambey 21).

Table 3. NCBI database Blast of DNA sequences produced by the SSR-6258 marker (related to photosensitivity) in photosensitive and non-photosensitive varieties.

Character	Varieties	Corresponding DNA sequences or proteins	Max score	Total score	E value	Query cover (%)	Per. Ident (%)
Photosensibles	Ndout yellow pods, Ndout purple pods, Baye Ngagne	<i>V. unguiculata</i> cultivar Xiabao 2 chromosome Vu04	375	1407	6.10 ⁻¹⁰⁰	97	96.88
		predicted: <i>V. angularis</i> receptor-like protein 12 (LOC108344195), mRNA	178	424	2.10 ⁻⁴⁰	96	86.14
Non photosensible	Bambey 21	<i>V. unguiculata</i> cultivar Xiabao 2 chromosome Vu04	405	887	6.10 ⁻¹⁰⁹	96	95.98
		predicted: <i>V. angularis</i> receptor-like protein 12 (LOC108344195), mRNA	167	413	3.10 ⁻³⁷	96	85.62

Per. Ident: percentage identification.

not previously reported. However, Jeong and Clark (2005) hypothesized that there may be a relationship between CALVATA and flower receptor kinases (FRK) in the initiation of flowering

in photosensitive plants. Furthermore, it was shown by Bassett et al. (2000) in *Ipomoea nil*, an SD photosensitive plant, that an LRR-RLK, *I. nil* receptor protein kinase 1 (INRPK1), is produced

in higher amounts under SD conditions.

Jeong and Clark (2005) suggest a possible relationship between CLV1 and photosensitivity. However, the transition from SAM to flower

Table 4. Blast of the sequences of the 9 other SSR markers related to photosensitivity according to the BSA, in the NCBI database.

Markers code	Corresponding DNA sequences or proteins	Max Score	Total Score	E value	Query cover (%)	Per. Ident (%)
SSR-6284	<i>V. unguiculata</i> cultivar Xiabao 2 chromosome Vu03	44.1	257	0.034	100	100
SSR-6292	<i>V. unguiculata</i> cultivar Xiabao 2 chromosome Vu06	40.1	110	0.53	100	100
SSR-6302	<i>V. unguiculata</i> cultivar Xiabao 2 chromosome Vu05	44.1	74.3	0.034	100	100
SSR-6304	<i>V. unguiculata</i> cultivar Xiabao 2 chromosome LG9	42.1	42.1	0.13	100	100
SSR-6314	<i>V. unguiculata</i> cultivar Xiabao 2 chromosome Vu05	44.1	74.3	0.034	100	100
SSR-6323	<i>V. unguiculata</i> cultivar Xiabao 2 chromosome Vu09	40.1	40.1	0.53	100	100
	<i>V. unguiculata</i> ubiquitin-conjugating enzyme E2 28-like (LOC114163067), mRNA	40.1	40.1	0.53	100	100
	<i>Asparagus officinalis</i> ubiquitin-conjugating enzyme E2-17 kDa-like (LOC109850825), transcript variant X3, mRNA	40.1	40.1	0.93	100	100
	<i>Coprinosia cinerea</i> okayama7#130 ubiquitin-conjugating enzyme E2 (CC1G_11074), mRNA	40.1	40.1	0.93	100	100
SSR-6531	<i>V. unguiculata</i> cultivar Xiabao 2 chromosome Vu05	40.1	134	0.53	100	100
SSR-6876	<i>V. unguiculata</i> cultivar Xiabao 2 chromosome Vu05	42.1	263	0.13	100	100
SSR-6906	<i>V. unguiculata</i> cultivar Xiabao 2 chromosome Vu04	38.2	159	2.1	95	100

Per. Ident: percentage identification.

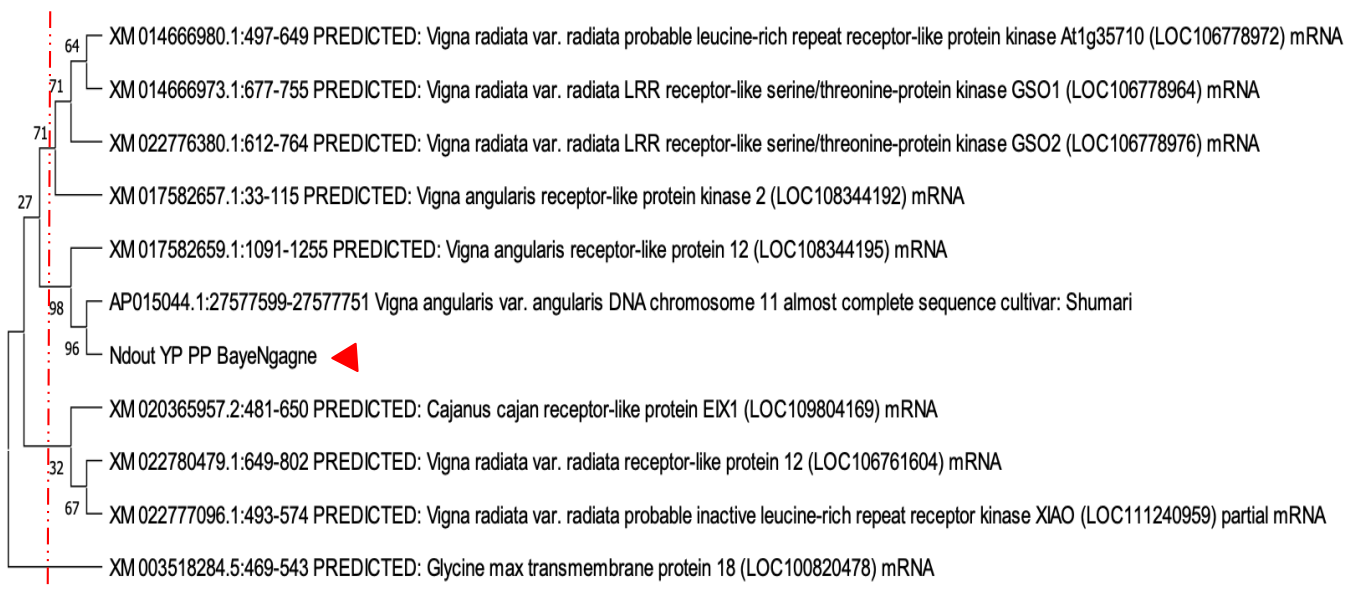
PREDICTED: Vigna angularis receptor-like protein 12 (LOC108344195), mRNASequence ID: [XM_017582659.1](#) Length: 2544 Number of Matches: 3Range 1: 1091 to 1255 [GenBank](#) [Graphics](#)[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
178 bits(96)	2e-40	143/166(86%)	2/166(1%)	Plus/Minus
Query 1	GGTTTCCTAGTTGGGAAGGAATGTATCCTTCAAAGAATTCTAG-TGAGATCAAGATAC	59		
Sbjct 1255	GGTTTCCAAGTTGGGAAGGAATGAATCCTTCAAAGAATT-GTAGCTGAGATCAAGATAC	119'		
Query 60	TGCAACCGAGAGAGATTTCCAATCTGAGATGGGAACTTCCTTCAAAGAATTCCCACTG	119		
Sbjct 1196	TGCAACCGAGAGAGATTTCCAAGTTGGGAAAGTATATTTCCATTAAAGAATTGCCACTG	113'		
Query 120	AGGTCAAGATACTGCAACGAGAGAGATTTCCAATCTGAGATGGGA	165		
Sbjct 1136	AGATCAAGATACTGCAACCGAGAGAGATTTCCAAGTTGAGGAGGGA	1091		

Figure 4. Alignment in the NCBI database of the sequence related to photosensitivity, produced by the photosensitive varieties (Ndout YP, PP, and Baye Ngagne).

PREDICTED: *Vigna angularis* receptor-like protein 12 (LOC108344195), mRNASequence ID: [XM_017582659.1](#) Length: 2544 Number of Matches: 3Range 1: 1097 to 1255 [GenBank](#) [Graphics](#)[▼ Next Match](#) [▲ Previous](#)

Score	Expect	Identities	Gaps	Strand
167 bits(90)	3e-37	137/160(86%)	2/160(1%)	Plus/Minus
Query 1	GGTTTCCTAGTTGGGAAGGAATGTATCCTTCAAAAGAATTCCCAG-TGAGATCAAGATAC	59		
Sbjct 1255	GGTTTCCTAGTTGGGAAGGAATGTATCCTTCAAAAGAATT-GTAGCTGAGATCAAGATAC	119		
Query 60	TGCAACCGAGAGAGATTTCCTTCAAAAGAATTCCCAG-TGAGATCAAGATAC	119		
Sbjct 1196	TGCAACCGAGAGAGATTTCCTTCAAAAGAATTCCCAG-TGAGATCAAGATAC	113		
Query 120	AGATCAAGATACTGCAAGAGAGAGAGATTTTCAATCTGAG	159		
Sbjct 1136	AGATCAAGATACTGCAACCGAGAGAGAGATTTTCAATCTGAG	1097		

Figure 5. Alignment in the NCBI database of the sequence related to photosensitivity, produced by the non-photosensitive variety (Bambey21).**Figure 6.** Dendrogram illustrating the relationship between DNA sequences linked to photosensitivity of Ndout YP, PP and Baye Ngane varieties and the probable proteins.

involves several genes, including CLV1, 2, 3, and the WUSCHEL (WUS) gene. Indeed, in the MAS, the expression of WUS leads to the maintenance of the MAS and the absence of flowering, whereas its repression by the interaction of CLV3, CLV1, CLV2, and the SUPPRESSOR OF LLP12 (SOL2)/CORYNE (CRN) causes cell proliferation, promoting their differentiation, leading to flowering (Clark et al., 1993; Laux et al., 1996; Kayes and Clark, 1998; Fletcher et al., 1999; Schoof et

al., 2000; Bassett et al., 2000; Brand et al., 2000; Kinoshita et al., 2010). CLV2 from *Arabidopsis* has been shown to encode a leucine-rich repeat (LRR) receptor-like protein (RLP) that lacks a cytoplasmic kinase domain (Laux et al., 1996; Shiu and Bleecker, 2001; Somssich et al., 2016). This study's results from alignments performed in NCBI revealed that the sequence related to photosensitivity corresponds to 86.14% to a portion of the RLP12 gene (Table 3 and

Figure 4). Also, the relationship of this sequence with RLP12 was confirmed by the dendrogram (Figure 6), clustering them in the same subgroup.

In light of all these observations, it could be envisaged that light-sensitive cowpea varieties, possessing protein complexes (LRR-RLP and/or LRR-RLK) allowing the perception and transmission of extracellular signals, would be able to control their flowering by blocking the expression of their WUS genes via the CLV genes. Modification of these genes would lead to a loss of photosensitivity.

Sequence alignment of the other 9 primers linked to photosensitivity revealed that the SSR-6323 marker is perfectly identical to part of the E2 28-like ubiquitin conjugating enzyme (Table 4). The enzymes (E2), with E1 and E3, constitute the trio of enzymes responsible for cellular protein ubiquitination (Jeong et al., 1999; Stewart et al., 2016). These enzymes act in cascade with the first activation of ubiquitin by E1, then its transfer to E3 is ensured by E2, and E3 transfers ubiquitin to the protein destined for degradation (Dye and Schulman, 2007; Lee and Schindelin, 2008; Choo and Zhang, 2009; Hennell et al., 2017). The E3 enzyme is encoded by the *constitutively photomorphogenic 1 (COP1)* gene, which is involved in several light-induced developmental processes (circadian rhythm and flowering) in *Arabidopsis* and other higher plants (Yu et al., 2008; Lau and Deng, 2012; Huang et al., 2014; Xu et al., 2016). In *Arabidopsis*, COP1 works in conjunction with the phyA-105 protein suppressor (SPA) to target photomorphogenesis factors for degradation via the 26S proteasome system during the SD period and prevent flowering (Laubinger et al., 2006; Jang et al., 2008; Zhu et al., 2008; Xu et al., 2016). The orthologue of COP1 in *Oryza* is Peter Pan syndrome (PPS), which controls photoperiodic flowering through the heading date 1 (Hd1) gene, which inhibits photomorphogenesis factors during the long day period (Tsuge et al., 2001; Ordoñez-Herrera et al., 2018). This work has shown that the photosensitivity band (289 bp) is longer than that produced in the non-photosensitive variety (Figure 1C). It was deduced that this deletion would have modified the 28-like ubiquitin E2-conjugating enzyme gene, rendering it unable to inhibit morphogenesis factors in non-photosensitive varieties, which would make their flowering independent of photoperiod. Whereas in the light-sensitive varieties, this gene would have undergone no modification, which would maintain the cascade function of the E1, E2, and E3 enzymes in long day and their non-flowering.

Conclusion

This work aimed to identify molecular markers associated with photoperiod sensitivity. Using the BSA technique, 10 markers related to photoperiod sensitivity were identified. Among these markers, the SSR-6258

marker was used to characterize 22 cowpea varieties with known photosensitivity, reproducing the photosensitivity band only in the photosensitive varieties. The sequence of this band is similar in the photosensitive varieties but differs by 28% from that produced in the non-photosensitive variety. Alignment of this sequence in the NCBI databases showed that 86.14% of it corresponds to the part of the CLV2 gene involved in SAM differentiation. Alignment of the sequences of the other primers associated with photosensitivity showed that the sequence of the SSR-6323 marker corresponds to part of the E2 gene, which is involved in the ubiquitination of E3, which is involved in photosensitivity. This marker could be used as a new resource in variety selection for photoperiod sensitivity, and its relationship with the marker SSR-6323 should be determined. This study's results provide probable relationships between a DNA fragment from photosensitive cowpea varieties and LRRs (RLP) and RLK. Their functions in cowpea should be studied in the future to determine their precise relationship with photosensitivity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

- Badiane FA, Gowda BS, Cissé N, Diouf D, Sadio O, Timko MP (2012). Genetic relationship of cowpea (*Vigna unguiculata*) varieties from Senegal based on SSR markers. *Genetics and Molecular Research* 11(1):292-304.
- Barakat MN, Al-Doss AA, Elshafei AA, Moustafa KA (2012). Bulk segregant analysis to detect quantitative trait loci (QTL) related to heat tolerance at grain filling rate in wheat using simple sequence repeat (SSR) markers. *African Journal of Biotechnology* 11(61):12436-12442.
- Bassett CL, Nickerson ML, Cohen RA, Rajeevan MS (2000). Alternative transcript initiation and novel post-transcriptional processing of a leucine-rich repeat receptor-like protein kinase gene that responds to short-day photoperiodic floral induction in morning glory (*Ipomoea nil*). *Plant Molecular Biology* 43(1):43-58.
- Brand U, Fletcher JC, Hobe M, Meyerowitz EM, Simon R (2000). Dependence of stem cell fate in *Arabidopsis* on a feedback loop regulated by CLV3 activity. *Science* 289:617-619.
- Bressani R (1985). Nutritive value of cowpea. *Cowpea Research, Production and Utilization* 353-359.
- Choo YS, Zhang Z (2009). Detection of Protein Ubiquitination. *Journal of Visualized Experiments* (30):1293.
- Clark SE, Running MP, Meyerowitz EM (1993). CLAVATA1, a regulator of meristem and flower development in *Arabidopsis*. *Development* 119(2):397-418.
- Clark SE, Williams RW, Meyerowitz EM (1997). The CLAVATA1 gene

- encodes a putative receptor kinase that controls shoot and floral meristem size in *Arabidopsis*. *Cell* 89(4):575-585.
- Dye BT, Schulman BA (2007). Structural mechanisms underlying posttranslational modification by ubiquitin-like proteins. *Annual Review of Biophysics and Biomolecular Structure* 36(1):131-150.
- Ehlers J, Hall A (1996). Genotypic classification of cowpea based on responses to heat and photoperiod. *Crop Science* 36(3):673-679.
- Food and Agriculture Organization of the United Nations (FAO) (2010). Food and agriculture data. <https://www.fao.org/faostat/en/#home>.
- Farooqi MQU, Sa KJ, Hong TK, Lee JK (2016). Bulk segregant analysis (BSA) for improving cold stress resistance in maize using SSR markers. *Genetics and Molecular Research* 15(4):10-4238.
- Felsenstein J (1985). Confidence Limits on Phylogenies with a Molecular Clock. *Systematic Zoology* (34):152-161.
- Fletcher JC, Brand U, Running MP, Simon R, Meyerowitz EM (1999). Signaling of cell fate decisions by CLAVATA3 in *Arabidopsis* shoot meristems. *Science* 283(5409):1911-1914.
- González AM, Yuste-Lisbona FJ, Weller J, Vander Schoor JK, Lozano R, Santalla M (2021). Characterization of QTL and Environmental Interactions Controlling Flowering Time in Andean Common Bean (*Phaseolus vulgaris* L.). *Frontiers in Plant Science* 11:599462.
- Gueye M (1983). *Vigna unguiculata* en symbiose avec Rhizobium et Glomus mosseae. ORSTOM Paris.
- Hadley P, Roberts EH, Summerfield RJ, Minchin FR (1983). A Quantitative Model of Reproductive Development in Cowpea [*Vigna unguiculata* (L) Walp.] in relation to Photoperiod and Temperature, and Implications for Screening Germplasm. *Annals of Botany* 51(4):531-543.
- Hall AE, Cisse N, Thiaw S, Elawad HO, Ehlers JD, Ismail AM, McWatters KH (2003). Development of cowpea cultivars and germplasm by the Bean/Cowpea CRSP. *Field Crops Research* 82(2-3):103-134.
- Hennell JR, Caceres EF, Escasinas A, Alhasan H, Howard JA, Deery MJ, Robinson NP (2017). Functional reconstruction of a eukaryotic-like E1/E2/(RING) E3 ubiquitylation cascade from an uncultured archaeon. *Nature Communications* 8(1):1120.
- Huang X, Ouyang X, Deng XW (2014). Beyond repression of photomorphogenesis: role switching of COP/DET/FUS in light signaling. *Current Opinion in Plant Biology* 21:96-103.
- Jang S, Marchal V, Panigrahi KCS, Wenkel S, Soppe W, Deng XW, Coupland G (2008). *Arabidopsis* COP1 shapes the temporal pattern of CO accumulation conferring a photoperiodic flowering response. *The EMBO Journal* 27(8):1277-1288.
- Jeong S, Clark SE (2005). Photoperiod Regulates Flower Meristem Development in *Arabidopsis thaliana*. *Genetics* 169(2):907-915.
- Jeong S, Trotochaud AE, Clark SE (1999). The *Arabidopsis* CLAVATA2 gene encodes a receptor-like protein required for the stability of the CLAVATA1 receptor-like kinase. *Plant Cell* 11(10):1925-1933.
- Kayes JM, Clark SE (1998). CLAVATA2, a regulator of meristem and organ development in *Arabidopsis*. *Development* 125(19):3843-3851.
- Kinoshita A, Betsuyaku S, Osakabe Y, Mizuno S, Nagawa S, Stahl Y, Sawa S (2010). RPK2 is an essential receptor-like kinase that transmits the CLV3 signal in *Arabidopsis*. *Development* 137(22):3911-3920.
- Lau OS, Deng XW (2012). The photomorphogenic repressors COP1 and DET1: 20 years later. *Trends in Plant Science* 17(10):584-593.
- Laubinger S, Marchal V, Gentilhomme J, Wenkel S, Adrian J, Jang S, Hoecker U (2006). *Arabidopsis* SPA proteins regulate photoperiodic flowering and interact with the floral inducer CONSTANS to regulate its stability. *Development* 133(16):3213-3222.
- Laux T, Mayer KF, Berger J, Jürgens G (1996). The WUSCHEL gene is required for shoot and floral meristem integrity in *Arabidopsis*. *Development* 122(1):87-96.
- Lee I, Schindelin H (2008). Structural insights into E1-catalyzed ubiquitin activation and transfer to conjugating enzymes. *Cell* 134(2):268-278.
- Michelmore RW, Paran I, Kesseli RV (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proceedings of the National Academy of Sciences* 88(21):9828-9832.
- Ndiaye M, Spencer M, Gueye M (2000). Genetic variability in dinitrogen fixation between cowpea [*Vigna unguiculata* (L.) Walp] cultivars determined using the nitrogen-15 isotope dilution technique. *Biology and Fertility of Soils* 32(4):318-320.
- Nielsen SS, Brandt WE, Singh BB (1993). Genetic Variability for Nutritional Composition and Cooking Time of Improved Cowpea Lines. *Crop Science* 33(3):469-472.
- Njoku E (1958). The photoperiodic response of some Nigerian plants. *Journal of the West Africa Science Association* 4:99-111.
- Nuhu Y, Mukhtar F (2013). Screening of some cowpea genotypes for photosensitivity. *Bayero Journal of Pure and Applied Sciences* 6(2):31-34.
- Ordoñez-Herrera N, Trimborn L, Menje M, Henschel M, Robers L, Kaufholdt D, Hoecker U (2018). The Transcription Factor COL12 Is a Substrate of the COP1/SPA E3 Ligase and Regulates Flowering Time and Plant Architecture. *Plant Physiology* 176(2):1327-1340.
- Ouédraogo J, Gowda B, Jean M, Close TJ, Ehlers JD, Hall AE, Belzile FJ (2002). An improved genetic linkage map for cowpea (*Vigna unguiculata* L.) combining AFLP, RFLP, RAPD, biochemical markers, and biological resistance traits. *Genome* 45(1):175-188.
- Poopprompan P, Wasee S, Toojinda T, Abe J, Chanprame S, Srinives P (2006). Molecular marker analysis of days to flowering in vegetable soybean (*Glycine max* (L.) Merrill). *Agriculture and Natural Resources* 40(3):573-581.
- Rasouli M, Fatahi R, Zamani Z, Imani A, Martínez-Gómez P (2013). Identification of simple sequence repeat (SSR) markers linked to flowering time in almond by bulked segregant analysis (BSA). pp 53-56.
- Schoof H, Lenhard M, Haecker A, Mayer KF, Jürgens G, Laux T (2000). The stem cell population of *Arabidopsis* shoot meristems is maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. *Cell* 100(6):635-644.
- Shiu SH, Bleecker AB (2001). Receptor-like kinases from *Arabidopsis* form a monophyletic gene family related to animal receptor kinases. *Proceedings of the National Academy of Sciences* 98(19):10763-10768.
- Singh B, Ehlers J, Sharma B, Freire Filho F (2002). Recent progress in cowpea breeding. *FATOKUN, CA; TARAWALI, SA; SINGH, BB; KORMAWA, PM* 22-40.
- Somssich M, Je BI, Simon R, Jackson D (2016). CLAVATA-WUSCHEL signaling in the shoot meristem. *Development* 143(18):3238-3248.
- Stewart MD, Ritterhoff T, Kleivit RE, Brzovic PS (2016). E2 enzymes: more than just middle men. *Cell Research* 26(4):423-440.
- Suh JB, Simbi CO (1983). Studies on the Legume Pod-Borer, *Maruca Testulalis* (Geyer)—VIII. Cowpea Phenology and Yield Loss Assessment: Effect of Loss of Leaves, Shoots, Flowers and Pods on Cowpea Yield in Western Kenya. *International Journal of Tropical Insect Science* 4(1-2):89-96.
- Sun F, Xu M, Park C, Dwiyantri MS, Nagano AJ, Zhu J, Abe J (2019). Characterization and quantitative trait locus mapping of late-flowering from a Thai soybean cultivar introduced into a photoperiod-insensitive genetic background. *PLoS One* 14(12):e0226116.
- Tamura K, Stecher G, Kumar S (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution* 38(7):3022-3027.
- Tarawali S, Singh B, Peters M, Blade S (1997). Cowpea haulms as fodder. *Advances in Cowpea Research* 313.
- Timko MP, Rushton PJ, Laudeman TW, Bokowiec MT, Chipumuro E, Cheung F, Chen X (2008). Sequencing and analysis of the gene-rich space of cowpea. *BMC Genomics* 9(1):103.
- Tsuge T, Inagaki N, Yoshizumi T, Shimada H, Kawamoto T, Matsuki R, Matsui M (2001). Phytochrome-mediated control of COP1 gene expression in rice plants. *Molecular Genetics and Genomics* 265(1):43-50.
- Wang F, Li S, Kong F, Lin X, Lu S (2023). Altered regulation of flowering expands growth ranges and maximizes yields in major crops. *Frontiers in Plant Science* 14:1094411.
- Wang F, Liu S, Li H, Fang C, Fang S, Wang J, Lin X (2024). Artificial selection of two antagonistic E3 ubiquitin ligases finetunes soybean

- photoperiod adaptation and grain yield. Proceedings of the National Academy of Sciences 121(45):e2321473121.
- Xu D, Zhu D, Deng XW (2016). The role of COP1 in repression of photoperiodic flowering. F1000 F1000Research 5:F1000 Faculty Rev-178.
- Yu JW, Rubio V, Lee NY, Bai S, Lee SY, Kim SS, Deng XW (2008). COP1 and ELF3 control circadian function and photoperiodic flowering by regulating GI stability. Molecular Cell 32(5):617-630.
- Zhang G, Chen L, Xiao G, Xiao Y, Chen X, Zhang S (2009). Bulk segregant analysis to detect QTL related to heat tolerance in rice (*Oryza sativa* L.) using SSR markers. Agricultural Sciences in China 8(4):482-487.
- Zhu D, Maier A, Lee JH, Laubinger S, Saijo Y, Wang H, Deng XW (2008). Biochemical Characterization of Arabidopsis Complexes Containing CONSTITUTIVELY PHOTOMORPHOGENIC1 and SUPPRESSOR OF PHYA Proteins in Light Control of Plant Development. Plant Cell 20(9):2307-2323.