Characterization of a dominant *SmNac-like* gene as a candidate for photosensitivity in the fruit peel of eggplant

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Running title: *SmNac-like* gene controls photosensitivity trait in eggplant

ABSTRACT

Anthocyanins in the fruit peel of photosensitive eggplants exhibit a different distribution pattern compared to the photo-insensitive ones. The latter exhibits a uniform anthocyanin content, whereas photosensitive eggplants lack anthocyanin accumulation in areas not exposed to light, such as under the calyx, or have lower concentrations in less-exposed areas. In the current research work, genetic analysis of F1 and F2 populations revealed that the non-photosensitive phenotype in eggplants follows an autosomal dominant inheritance with a 3:1 ratio, indicating that the photosensitive trait is regulated by a single dominant gene. To locate and narrow down the position of the gene underlying photosensitivity, an F2 population segregating for photosensitivity and presence of anthocyanins was used for bulked segregant analysis sequencing (BSA-seq), and RNA-seq analysis of bagged photosensitive and non-photosensitive eggplants was performed. As a result, we identified the *SmNAC1-like* gene as a candidate for fruit photosensitivity trait in the eggplant materials used in this study. In the photo-insensitive eggplants (DH-ECAVI and IVIA-371) two different high-impact mutations were identified leading to light-insensitive and uniform anthocyanin content. The mutation in DH-ECAVI causes a premature stop codon, while in IVIA-371 an indel mutation occurs in the 3'-UTR sequence. No high-impact mutations were found in *SmNAC1-like* gene in photo-sensitive eggplants. These findings provide new insight into the regulation of the molecular mechanisms of anthocyanin biosynthesis in non-photosensitive eggplant and have important implications for marker-assisted selection and breeding.

KEYWORDS

Solanum melongena, *SmNAC1-like*, Anthocyanin Biosynthesis and Photosensitivity

INTRODUCTION

Eggplant (*Solanum melongena* L.) is a solanaceous crop with high economic value and characterized by the presence of high concentrations of anthocyanins in the fruit peel of some cultivars. Anthocyanins are water-soluble pigments derived from anthocyanidins by the addition of sugars, which make flowers and fruits appear red, blue or purple (Takos et al., 2006 and Mattioli et al., 2020). In addition, anthocyanins offer several benefits to plants, such as protection against UV radiation, extreme temperatures, and pathogen attacks (Lorenc-Kukula et al., 2005; Liu et al., 2013 and Ren et al., 2014). Finally, anthocyanins contribute to human health, since they present strong antioxidant properties, with beneficial effects on human well-being (Muñoz-Falcón et al., 2009; Speer et al., 2020).

Light plays a major role in the synthesis of anthocyanins in the fruit peel of eggplants. Previous research in photosensitive eggplants demonstrated that light activates the anthocyanin biosynthetic pathway leading to the accumulation of anthocyanins in the fruit peel in a time-exposure manner (Jiang et al., 2016; Li et al., 2017; Sang et al., 2022, Li et al., 2023). However, non-photosensitive eggplants are able to synthesize large amounts of anthocyanins under shaded conditions (Jiang et al., 2016 and Sang et al., 2022). A special case is the fruit peel under the eggplant calyx, which is always shaded and therefore remains uncolored (either partially or completely) in photosensitive fruit peels compared to non-photosensitive ones, indicating that light is required for the development of purple pigmentation in eggplant (Li et al., 2017; Li et al., 2018 and Zhang et al., 2019). Consequently, breeding for non-photosensitive eggplant varieties is an effective method to maintain a stable anthocyanin accumulation independently of environmental conditions. Given the relevance of the non-photosensitivity trait in eggplant breeding, some studies have focused on mapping the genes responsible for photoinsensitivity. These studies have identified different QTLs associated with non-photosensitivity across different chromosomes, likely due to the use of diverse eggplant materials, research backgrounds and eggplant reference genomes (Qiao et al., 2022; He et al., 2022; Mangino et al., 2022; Luo et al., 2023a-2023b and You et al., 2023). Several genes are involved in the regulation of anthocyanin biosynthesis in eggplant where the structural genes in the anthocyanin biosynthetic pathway include enzyme-encoding genes such as *CHS*, *CHI*, *F3H*, *F3'H*, *F3'5'H*, *DFR* and *ANS* (Jaakola et al., 2013 and Liu et al., 2018).

76 Those genes are regulated by transcription factors (TFs), several of which have been postulated as
77 candidates to regulate anthocyanin gene expression (Li et al., 2017; Li et al., 2018; Qiao et al., 2022;
78 He et al., 2022; Mangino et al., 2022; Zhou et al., 2022; Li et al., 2023; Luo et al., 2023a-2023b You et
79 al., 2023). A common feature of the candidate TFs is that they activate R2R3-MYB, basic helix-loop-
80 helix (bHLH) or WD repeat proteins (WDR), which form the MYB-bHLH-WDR (MBW) complex to
81 regulate anthocyanins structural biosynthesis genes (Ramsay et al., 2005; Yan et al., 2021). Researching
82 features connected to anthocyanins fruit peel pigmentation can help us comprehend this characteristic
83 of eggplants and create new kinds of eggplants with varied anthocyanin coloration.
84 In this study, we investigated the photosensitivity trait in local eggplant varieties from País Valencià.
85 To map non-photosensitivity trait, we evaluated an F2 population derived from a cross between a
86 photosensitive cultivar (*S. melongena* ASI-S-1) and a non-photosensitive variety (*S. melongena* IVIA-
87 371). This population also segregates for anthocyanin and clorophyll pigmentation. Bulked segregant
88 analysis by sequencing (BSA-seq), combined with RNA-seq, was applied to identify candidate loci,
89 *SmNAC-like protein,* for photosensitivity (PS) in the F2. The presence of DNA mutations in the locus
90 identified was in agreement with the genotype of non-photosensitive phenotypes (e.g., IVIA-371 and
91 DH-ECAVI) and photosensitive phenotypes (e.g., AN-S-26, H15, and ASI-S-1). Two different
92 mutations in the down-regulated *SmNac1-like* gene, a transcription factor, were found in IVIA-371 and
93 DH-ECAVI but not in the photosensitive accessions. The identification of *SmNac1-like* as a candidate
94 gene for the eggplant photosensitive trait is useful to understand the genetic basis of the regulation of
95 the anthocyanin pigmentation in the eggplant fruit peel, as well as for eggplant breeding.
96

97 **MATERIAL AND METHODS**

98 **1.- Plant materials and cultivation conditions.**

99 The *S. melongena* accessions ASI-S-1 (anthocyanin photosensitive, non-striped and with presence of
100 chlorophyll pigmentation in the peel) and IVIA-371 (anthocyanin non-photosensitive, striped and no
101 chlorophyll pigmentation in the peel) were used as parents to develop an F2 population of 120
102 individuals segregating for the photosensitivity trait (PS), presence of fruit anthocyanin (FA) and
103 chlorophyll content (CC) (Figure 1 Panels A and B). PS was categorized as PUC (presence of purple
104 pigmentation under the calyx, i.e., negative for PS) or puc (absence of purple pigmentation under the
105 calyx, i.e., positive for PS. In addition to the eggplant accessions ASI-S-1 and IVIA-371, two sub-
106 introgression lines (IL-69(6)5-12-Ch10 and IL-69(6)5-8-Ch10, derived from the introgression line IL-
107 M5-19) plus accessions DH-ECAVI (photoinsensitive-anthocyanin uniform pigmentation), H15 and
108 AN-S-26 (photosensitive- anthocyanins in the fruit peel exposed to sunlight) were used in the current
109 study (Mangino et al., 2022). With regards to the phenotype of IL-69(6)5-12-Ch10 and IL-69(6)5-8-
110 Ch10 showed a complete loss of anthocyanins content in the fruit peel (Figure S1). The introgression
111 line IL-M5-19 introgression line derives from the MEL5 eggplant (photosensitive) with an introgression
112 from the eggplant wild relative *S. insanum* in chromosome 10.

113 Seeds of all the accessions were germinated in Petri dishes following the Ranil et al. (2015) protocol
114 and transferred to seedling trays in a climatic chamber under a photoperiod 16 h light/ 8 h dark and
115 temperature regime of 22 ± 2 ºC. The plantlets were grown for four weeks (up to the fifth true leaf
116 development stage) in a climatic chamber before acclimatization for 48 hours in a benched glasshouse.
117 Plantlets were then transplanted to 15 L pots filled with coconut fiber and grown in a pollinator-free
118 benched glasshouse of the Universitat Politècnica de València (UPV), Valencia, Spain. Plants were
119 spaced 1.2 m between rows and 1 m within the row, fertirrigated using a drip irrigation system and
120 trained with vertical strings. Pruning was done manually to regulate vegetative growth and flowering.
121 Phytosanitary treatments were performed when necessary.

122 **2.- Generation of a F2 population and BSA-Seq analysis of PUC vs puc bulks.**

123 The F2 population obtained by the intraspecific cross between ASI-S-1 and IVIA-371 accessions was
124 phenotyped for the PS and FA fruit traits. A Chi-square test was performed to assess the goodness-of-

125 fit to a monogenic dominant inheritance pattern (3:1 segregation model). The F2 population was used
126 for a BSA-Seq for the PS trait. The PS have been scored in 94 plants out of the 120 of the total
127 population, as 26 plants did not display anthocyanin pigmentation. The PUC and puc bulks consisted
128 of 15 F2 individuals. Young leaves were harvested from the entire F2 population (120 F2 individuals)
129 as well as from six individual plants for each of the two parents. Their DNA was isolated, quantified
130 and tested for quality prior to select six plants from each of the parents and 30 individual F2 plants
131 based on their different PS phenotype (PUC vs. puc). Genomic DNA was extracted using the silica
132 matrix (SILEX) protocol (Vilanova et al., 2020) and checked for integrity by agarose electrophoresis.
133 NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA) ratios 260/280 and 260/230 were used to
134 test the DNA quality, while its concentration was estimated with a Qubit 2.0 Fluorometer (Thermo
135 Fisher Scientific, Waltham, MA, USA).
136 Pools for the PUC and puc phenotypes bulks were made by mixing in a equimolecular from each of
137 selected F2 individual and shipped to Novogene (Novogene Europe, Cambridge, United Kingdom)
138 where genomic libraries (PE 150, insert 350 bp) were constructed and sequenced. All raw reads were
139 trimmed using the fastq-mcf tool from the Ea-utils package (Aronesty, 2013) with "q 30 -l 50"
140 parameters, and the overall quality was checked using FastQC v.0.12.1 (Andrews et al., 2010). Clean
141 reads were mapped against the 67/3 v3 (for QTL comparison and DNA variant analysis in reported
142 eggplant populations with PS trait) and v.4.1 (for data cross-referencing with RNA-seq) eggplant
143 reference genome (Barchi et al., 2019). To map the clean reads BWA-MEM v.0.7.17–r1188 was used
144 with default parameters (Li and Durbin et al., 2009). The ΔSNP-index was estimated using the QTL-
145 seq software v.2.2.3 (Takagi et al., 2013) from BAM files.

146 **3.- RNA-seq analysis of F2 population.**

147 RNA-seq analysis was conducted on bagged fruits from the F2 population to ascertain the gene
148 expression profile of eggplant fruits with and without PS. Eggplant flowers from each plant were
149 manually pollinated and kept in opaque paper bags for five days in the dark. The time lapse was selected,
150 as it was found to be the shortest time for the appearance of anthocyanin pigmentation. Subsequently,
151 a set of eggplant fruit peels was gathered by cutting them with a surgical blade avoiding as much as

152 possible to collect eggplant flesh, and subsequently frozen in liquid nitrogen before being kept in storage
153 at -80ºC. The eggplant fruit peels were grouped in three randomized replicates for each phenotype of
154 PS trait (PUC and puc). For the PUC samples, each replicate consisted of 4 fruit peels. However, for
155 the puc samples, due to the limitation in the number of fruits available, each replicate consisted of three
156 samples. The RNA extractions were done with RNAqueous kit and Ambion Plant RNA isolation aid
157 reagent (Invitrogen™ AM1912 and AM6990) following the manufacturer instructions. The RNA
158 integrity and quality were checked by 1.5% agarose gel and with the spectrometer Nanodrop 1000
159 (Thermo Scientific). RNA concentration was measured using a Qubit 2.0 Fluorometer (Invitrogen) and
160 an AccuBlue ® Broad Range RNA quantitation kit (Biotium). The samples with the highest values of
161 260/280 and 260/230 ratios were selected, normalized for their concentration and randomly pooled.
162 RNA samples were shipped to Novogene Europe (Cambridge, UK) for library preparation and
163 sequencing. Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads.
164 Then, mRNA was fragmented and the first strand cDNA was synthesized using random hexamer
165 primers, followed by the second strand cDNA synthesis. After adapter ligation, fragments were size-
166 selected, PCR-amplified, and purified. The library was checked with Qubit and real-time PCR for
167 quantification and bioanalyzer for size distribution detection. Quantified libraries were pooled and
168 sequenced on Illumina platforms (NovaSeq 6000), according to effective library concentration and data
169 amount (9 GB of data). With regards to RNA-seq read processing, clean reads were obtained by
170 removing reads containing adapter, reads containing ploy-N and low-quality reads from raw data
171 through Novogene in-house perl scripts and Q20, Q30 and GC content were calculated. All the
172 downstream analyses were based on clean, high-quality data (Table S1). Reference genome (*S.*
173 *melongena* 67/3 v4.1) and gene model annotation files were downloaded from Sol Genomics Network
174 (SGN) website. Index of the reference genome was built and paired-end clean reads were aligned to the
175 reference genome using Hisat2 v2.0.5 (Zhang et al., 2021). This tool was selected for mapping as it can
176 generate a database of splice junctions based on the gene model annotation file, giving a better mapping
177 result than other non-splice mapping tools. Reads numbers mapped to each gene were counted using
178 featureCounts v1.5.0-p3 (Liao et al., 2014). Then, FPKM was calculated based on the length of each
179 gene and reads mapped to it.

180 Differential expression analysis was performed using the DESeq2 R package (1.20.0), which uses a
181 statistical model based on the negative binomial distribution for determining differential expression
182 (Love et al., 2014). The resulting P-values were adjusted using the Benjamini and Hochberg's approach
183 for controlling false discovery rate (Benjamini and Hochberg., 1995). Genes with an adjusted P-value
184 ≤ 0.05 were assigned as differentially expressed. Finally, Gene Ontology (GO) enrichment analysis of
185 differentially expressed genes (DEG) was implemented by the clusterProfiler R package (Yu et al.,
186 2012; Wu et al., 2021; Xu et al., 2024), which corrects gene length bias. GO terms with corrected P-
187 value < 0.05 were considered significantly enriched by differentially expressed genes. The
188 clusterProfiler R package was used to test the statistical enrichment of differential expression genes in
189 KEGG pathways database (http://www.genome.jp/kegg/).

190 **4. Evaluation of DNA sequence variants in photo-sensitive and photo-insensitive cultivars**
191 **from different eggplant population.**

192 To determine the presence of DNA sequence variants in the DEGs located inside the QTL identified at
193 chromosome 10 at photo-in/sensitive eggplant cultivars analyzed against v4.1 eggplant reference
194 genome (Barchi et al., 2019), firstly the genomic DNA chromosome 10 (63.4 – 67.3 Mb) from the
195 eggplant reference genome "67/3" v4.1 versus v3 were compared with D-Genies (Cabanettes, Klopp.,
196 2018). As a result, no major breaks and inversions were detected. So, the gene synteny remains constant
197 at the genomic DNA region under consideration. Secondly of all the clean reads of ASI-S-1 and IVIA-
198 371 obtained in a previously research (Mangino et al., 2022) was mapped against the eggplant reference
199 genome "67/3" v3 with BWA-MEM and only the uniquely aligned reads were selected. Regarding the
200 variant calling was carried out again, due that the reported sequence changes reported by genes Mangino
201 et al. (2022) were not validated by Sanger Sequencing (Table S2). With regard the new variant calling
202 filters for the PS trait in the F2 interspecific cross between ASI-S-1 and IVIA-371 were done with
203 FreeBayes (Garrison and Marth., 2012) with the following parameters: --min coverage 10, --limit
204 coverage 60 amd –min alternate fraction 0.2. The predicted effects of the sequence changes detected
205 were evaluated with SnpEff (Cingolaniet et al., 2012). The DNA variants that affect the regulatory
206 sequences (5'-UTR and 3'-UTR) and/or the coding sequences (i.e., frameshift, indels, splicing variants,

207 etc) for the PS trait in homozygous allelic variants were classified as “high impact”. The Integrative
208 Genomics Viewer (IGV) tool (Robinson et al., 2022) was used to validate the variants detected by the
209 combination of FreeBayes + SnpEff softwares. To expand the number of eggplants cultivars evaluated,
210 eggplants with PS trait (DH-ECAVI, ANS-S-26 and H15) previously reported (Mangino et al., 2022)
211 were evaluated. As previously indicated clean reads of the respective eggplants accessions was mapped
212 against the eggplant reference genome “67/3” v3 with BWA-MEM and only the uniquely aligned reads
213 were selected. Then the variant calling were done with FreeBayes (Garrison and Marth., 2012) with the
214 following parameters: --min coverage 10, --limit coverage 60 amd –min alternate fraction 0.2. The
215 predicted effects of the sequence changes detected were evaluated with SnpEff (Cingolaniet et al.,
216 2012). “High impact” variants were selected and validated with IGV
217

218 **RESULTS**

219 **1.- Identification and inheritance of a QTL linked to photoinsensitivity.**

220 **1.1.- Generation and inheritance of the scored traits in the F2 population.**

221 To determine the PS trait segregation pattern, an F2 population was produced by mating the *S.*
222 *melongena* parents ASI-S-1 (puc, non-striped, and with presence of chlorophyll pigmentation in the
223 peel) with IVIA-371 (PUC, striped, and with absence of chlorophyll pigmentation in the peel).
224 Regarding the presence of plant anthocyanin pigmentation in the parents, ASI-S-1 displayed
225 anthocyanin in leaves and stem, meanwhile IVIA-371 only showed anthocyanin content in the stem but
226 not in the leaves (Figure 1 Panel A). Regarding the F1, it displayed the PUC phenotype and FA
227 phenotype with uniform (non-striped) anthocyanin pigmentation and also showed background
228 chlorophyll presence in the peel. In the F2 generation, the PS, FA and CC traits segregated resulting in
229 several phenotypic combinations (Figure 1 Panel B) for the three traits present in the eggplant cultivars
230 used in this study.

231 The segregation pattern of the PS trait showed an autosomal dominant inheritance (Table 1), being
232 eggplants with PUC and non-striped phenotype the most abundant (Figure 1). Plants producing fruits
233 without anthocyanin pigmentation (i.e., green or white fruits), were not scored for the PS phenotypes
234 (as the phenotype could not be determined) (Figure 1 Panel B). These results suggested that the PS trait
235 in our IVIA-371 x ASI-S-1 population are controlled each by one dominant gene. On the other hand,
236 although the FA and CC traits segregate in the F2 population were not used in this study as they were
237 independent traits in relation to the PS trait (data not shown).

238 **1.2.- Identification of the genomic loci linked to PS trait by bulk segregation analysis in**
239 **the F2 population**

240 To narrow down the genomic loci linked to the PS trait, two DNA pools from the IVIA-371 x ASI-S-
241 1 F2 population (15 plants with the PUC phenotype and 15 with the puc phenotype) were sequenced.
242 This sequencing yielded an average of 154 million 150-bp raw reads per bulk, with a mean coverage
243 of 33.3x. Using ASI-S-1 as the reference sequence, the ΔSNP-index was calculated for 535,083 SNPs,
244 getting a QTL on chromosome 10 between 62.9-69.7 Mb with a 99 % confidence interval (CI) for the

245 PS trait. Similarly, when the ΔSNP-index was calculated for 562,169 SNPs using IVIA-371 as the
246 reference sequence, a region between 63,4-67,5 Mb was identified on the same chromosome 10 with a
247 99 % CI (Figure 2, Figure S2 and Table S3). As a result, 102 genes were identified within the
248 common candidate region (Table S4). Variant calling and prediction of the sequence changes were
249 evaluated in both candidate regions of ASI-S-1 and IVIA-371, no DNA variants in the coding
250 sequences (frameshift, indels, splicing variants, etc.) were detected**2.- Genetic evidences of QTl**
251 **for photoinsensitivity in different eggplant populations.**
252 In order to determine the relevance of the QTL on chromosome 10 in the photosensitivity trait, it was
253 compared with previous QTLs identified in different eggplant populations (Table 2). To be able to
254 compare with the previous data, the BSA-seq analysis was re-analyzed against the v3 of the eggplant
255 reference genome. As a result of the re-analysis a QTL in the chromosome 10 for ASI-S-1 between
256 84,1-87,9 Mb with 99 % CI and a region between 83,5-97,7 Mb with 95 % CI for IVIA-371 were
257 identified (Table S5, Table S6, Figure S2 and Figure S3). Firstly different sub introgression lines
258 derived from the IL-M5-19 introgression line (introgressed DNA of Solanum insanum into MEL5
259 eggplant) were evaluated. As a result, the sub introgression lines (IL-69(6)5-12-Ch10 and IL-69(6)5-8-
260 Ch10) with introgressed DNA at chromosome 10, IL-69(6)5-12-Ch10 at 84,4-102,8 Mb and IL-69(6)5-
261 8-Ch10 at 105,8-106,6 Mb respectively (Table 2), showed complete loss of anthocyanins content in the
262 fruit peel (Figure S1). With regards the S3MEGGIC population for FA (91.1-94.8 Mb) and PUC (91.1-
263 94.8 and 98.2-100.5 Mb) showed significant SNPs stacked at the end of chromosome 10 (Table 2).
264 Taking all the QTLs identified in the different populations together, suggest that the end of the
265 chromosome 10 as a hotspot related with anthocyanin related traits. Six of QTL reported showed
266 overlap (total or partial) along the different eggplant populations (Table 2). Of these six reported QTLs,
267 two groups can be observed: the first one consists of four overlapping QTLs (IL-69(6)5-12-Ch10 84,4-
268 102,8 Mb, FA 91.1-94.8 Mb, PUC 91.1-94.8 Mb, ASI-S-1 84,1-87,9 Mb and IVIA-371 83,5-97,7 Mb),
269 while the second one consists of two QTLs with partial overlap (IL-69(6)5-12-Ch10 at 84,4-102,8 Mb
270 and PUC 98.2-100.5 Mb). As a summary two common DNA regions across all the QTLs were identified
271 (QTLs_ASI-S-1 84.1-87.9 (3.80 Mb) and PUC 98.2-100.5 (2.3 Mb)).

272 **3.- Differential expression analysis of bagged eggplants**

273 The gene expression analysis between PUC and puc bagged eggplant fruits of F2 eggplant population
274 was performed by RNA-seq of the fruit peel. The results indicate that from a total of 177 DEGs, 53
275 DEGs were up-regulated in the fruit peel of eggplants with PUC phenotype, while 124 DEGs were
276 down-regulated in the fruit peel of eggplants with puc phenotype (Figure 3 and Table S7-9). The 177
277 DEGs identified from the eggplant fruit peel for the PS trait can be grouped in four different subclusters
278 (Table S9 and Figure S4). The subclusters 1 and 3 are made of the DEGs expressed mainly in IVIA-
279 371 (PUC phenotype) with a total of 53 genes, that constitute the whole up-regulated DEGs identified
280 by RNA-seq. The subclusters 2 and 4 contain 124 genes expressed totally in ASI-S-1 (puc phenotype),
281 and constitute all the down-regulated DEGs reported for the eggplant fruit peel (Table S2 and Table
282 S8). Concerning the functional and gene enrichment analysis of up- and down-regulated genes in
283 eggplant fruit peel with PUC and puc phenotypes, few DEGS were identified due to the fruit peel
284 characteristics. For the up-regulated DEGs expressed in IVIA-371, the gene set showed an enrichment
285 in KEGG terms related to flavonoid and phenylpropanoid biosynthesis (Table S7), mostly located in
286 the subcluster 1 (Table S8) and in agreement with the presence of anthocyanins in the fruit peel of
287 eggplant (Figure 3 and Table S8). The following up-regulated genes in flavonoid biosynthesis were
288 identified in IVIA-371: SMEL4.1_09g021180.1, SMEL4.1_05g002700.1, SMEL4.1_08g022350_1,
289 SMEL4.1_02g020840_1, SMEL4.1_05g002760_1, SMEL4.1_05g018170_1 and
290 SMEL4.1_12g016460_1. Regarding the GO annotation terms, an enrichment in terms like small
291 molecule metabolic process, transferase activity of acyl and hexosyl groups, or coenzyme bonding and
292 iron ion binding was observed. Those functions are consistent with the activity of the up-regulated genes
293 identified by the KEGG terms.

294 Concerning the down-regulated genes in IVIA-371 and, converserly, over-expressed in ASI-S-1, the
295 gene set showed an enrichment in KEGG terms related to MAPK signaling pathway, phenylpropanoid
296 biosynthesis, cysteine and methionine metabolism, zeatin and alanine biosynthesis, and aspartate and
297 glutamate metabolism (Table S7). The enrichment in those KEGG terms in the MAPK signaling
298 pathway and zeatin biosynthesis are involved in plant growth and fruit enlargement (Schäfer et al., 2015

299 and Jagodzik et al., 2018) in agreement with the fact that the eggplant fruits were growing. About the
300 KEGG terms of cysteine and methionine metabolism plus alanine, aspartate and glutamate metabolism
301 are reported to play a role in the generation of secondary metabolites involved in plant growth, fixing
302 inorganic sulfur, precursors of several biosynthesis pathways, and plant protection against abiotic stress
303 (Moormann et al., 2022). Finally, the accumulation of KEGG terms in phenylpropanoid biosynthesis
304 indicated an accumulation of the biosynthesis precursors of flavonoids (Vogt, 2010; Dong et al., 2021
305 and Liu et al., 2021). With respect to the GO annotation terms an enrichment in terms like coenzyme
306 binding, carbohydrate metabolic process, regulation of biological quality, or carbon-oxygen lyase
307 activity was observed. Those functions are consistent with the activity of the up-regulated genes
308 identified by the KEGG terms since they are involved in biosynthetic pathways.

309 **4.- Identification of genes expressed in photo-insensitive fruits in the dark conditions,**
310 ***SmNac1***

311 **4.1- Evaluation of the DEGs inside of the candidate region acotated in the F2 population**

312 To narrow down the number of putative candidate genes within the common candiate region of ASI-S-
313 1 and IVIA-371 (102 genes), their expression levels (Table S9) were evaluated given that no DNA
314 variant were found in the variant calling plus prediction of the sequence changes (see above – section
315 1.2). As a result, five genes were differentially expressed (*SMEL4.1_10g016630.1*,
316 *SMEL4.1_10g014730.1*, *SMEL4.1_10g013610.1*, *SMEL4.1_10g014530.1*, and
317 *SMEL4.1_10g019230.1*) (Table 3). The *SMEL4.1_10g016630.1* codifies for a CHI-like protein,
318 essential enzyme in the anthocyanin synthesis pathway, is up-regulated. This is in agreement with the
319 fact that the PUC and puc pools showed anthocyanin content in the fruit peel (Figure 2). With regards
320 the down-regulated genes (*SMEL4.1_10g014730.1*, *SMEL4.1_10g013610.1*, *SMEL4.1_10g014530.1*,
321 and *SMEL4.1_10g019230.1), SMEL4.1_10g014730.1* and *SMEL4.1_10g014530.1* codifies for a
322 NAC1-like protein (a transcription factor) RAD5B-like protein (DNA repair protein) respectively. The
323 remaining down-regulated genes encode proteins of unknown function (Table 3).

324 Since the differentially expressed genes did not show mutations in the coding sequences, the regulatory
325 regions (5'UTR and 3'UTR) were analysed. As a result the NAC-1 like protein (*SMEL4.1_10g014730.1*)

326 showed an indel in the 3'UTR (Reference: ATATTATTATTATTATTATC, Alternate variant:
327 ATATTATTATTATT---ATC) (File S1 and FileS2).

328 **4.2- Presence of DNA mutation of *SmNac1* in other eggplant accessions photosensible**
329 **(H15 and ANS-S-26) and photoinsenstible (DH-ECAVI)**

330 With the aim to known of the DNA mutation in the *SMEL4.1_10g014730.1* was present in other
331 eggplant accessions previously reported in the v3 genome eggplant consortium (Mangino et al., 2022).
332 Firstly the equivalent of the five genes differentially expressed were located in the v3 of the eggplant
333 refence genome. All of them were inside of the common DNA region of ASI-S-1 (84,1-87,9 Mb) and
334 IVIA-371 (83,5-97,7 Mb) (Table S6). Secondly the coding sequences,5'UTR and 3'UTR sequences
335 were reevaluated in photosensitivy (H15, ANS-S-26 and ASI-S-1) and photoinsensitivity (DH-ECAVI
336 and IVIA-371) eggplant accessions (Table 4).

337 As a result, in the photoinsensitivity eggplant accessions only *SMEL4.1_10g014730.1* showed the same
338 disruptive inframe insertion at 3'UTR in IVIA-371, plus DH-ECAVI showed a disruptive in frame
339 insertion that causes a frameshift in the coding sequence (Table 4 and File S1 and FileS2). With regards
340 the photosensitivy (H15, ANS-S-26 and ASI-S-1) eggplant no DNA mutations were detected in
341 *SMEL4.1_10g014730.1* and only for H15 *SMEL4.1_10g014530.1* showed a conservative in frame
342 insertion that do not change the open reading frame of RAD5B-like protein.

343 **DISCUSSION**

344 Plant domestication in the Solanaceae family resulted in a great diversity of fruit shapes, sizes, and fruit
345 colours. Despite this, modern breeding programs have generally favored eggplants with constant and
346 intense dark and purple pigmentation in the fruit peel (Samuels et al., 2015; Wang et al., 2015 and Taher
347 et al., 2017). In western markets, eggplants with black or purple peel are of higher economic value
348 (Muñoz-Falcón et al., 2009). In our work, different eggplant populations were used to evidence that
349 regarding to antocyanin related traits (complete absence of antocyanin, photsensitivy and fruit
350 anthocyanin) the chromosome 10 is a candidate DNA region to take into account. Anthocyanins have a
351 strong antioxidant effect, so their high content in the fruit peel of eggplant improves nutrition. So, a
352 research to determine the genetics basis of the PS is needed to set the basis of a new generation of
353 eggplants cultivars with high content of secondary metabolites beneficial for human health. In the
354 current research the PS showed an inheritance in agreement with a monogenic dominant gene, following
355 3:1 ratio in and F2 generation, consistent with previous research data (Honda et al., 2014; He et al.,
356 2022; Qiao et al., 2022; Luo et al., 2023ab). These eggplant accessions were selected because, based on
357 the corrected data reported by the GWAS study of the MAGIC population, neither of the two accessions
358 presented mutations in the CDS of the candidate regions, suggesting that expression must have been
359 altered. Regarding the fruit pigmentation of the F2 generation is probably due to the heterozygosity of
360 the parentals for the genes that controlled photosensitivity, plant anthocyanin, and fruit anthocyanin.

361 The reported studies on the PS trait used commercial varieties of eggplant (Honda et al., 2012) or non-
362 photosensitive eggplants varieties (He et al., 2022; Qiao et al., 2022; Luo et al., 2023ab) as parental
363 donors for the PUC phenotype. Meanwhile for the puc phenotype present in photosensitive eggplants
364 is recessive compared to the dominant allele of non-photosensitive eggplant varieties (Honda et al.,
365 2012; He et al., 2022; Qiao et al., 2022; Luo et al., 2023a and Luo et al., 2023b). It should be noted that
366 two commercial eggplant varieties with the PUC phenotype (IVIA-371) and (ASI-S-1) were used to
367 produce the F2 population.

368 As opposite what happens with other fruit colour traits (such as the green fruit neeting (Arrones et al.,
369 2022 and 2024, Toledo Garcia, et al., 2024)), that the phenotype present in the commercial eggplant
370 varieties involves loss of gene function and recessive inheritance, so the PS trait results in a dominant

371 inheritance and a gain of function. That phenomenon is quite uncommon since domestication usually
372 involves the loss of gene function or regulation (Lester and Hasan, 1991).
373 Regarding the localization of the reported QTLs (He et al., 2022; Qiao et al., 2022; Luo et al., 2023a,
374 Luo et al., 2023b) for the PS at the end chromosome 10 using the eggplant genome 67/3 v3 as a reference
375 with complete different eggplant accession is in agreement with our results, validating that region as a
376 promising localization to perform fine-tune mapping or expression analysis to identify gene candidates..
377 However, other QTLs for the PS trait have been reported in chromosome 1 and 3 (Mangino et al., 2022).
378 Here, we confirmed the end chromosome 10, using v3 eggplant genome as a reference, as candidate
379 region related with anthocyanin content traits, identifying 84 potential candidates genes for ASI-S-1
380 and 550 for IVIA-371. To filter the number of candidates genes identified by the BSA-seq analysis, the
381 list of genes was compared with the identified DEG from the RNA-seq of F2 population. Out of the
382 five genes identified as differentially expressed, the *SmNAC1-like* (SMEL4.1_10g014730.1) is mutated
383 in eggplant varieties with PUC phenotype (DH-ECAVI and IVIA-371) but not in puc phenotype (ANS-
384 S-26. H15 and ASI-S-1) eggplant varieties. All these results together provide strong evidence of
385 *SmNAC1-like* gene as candidate gene controlling the PUC trait in our eggplant varieties.
386 Regarding gene regulation of the anthocyanin biosynthetic pathway, previous studies suggest that light
387 and cold induce anthocyanin biosynthesis from the moment of exposure until saturation through a
388 highly dynamic range of TFs (Jiang et al., 2016; Li et al., 2017; Sang et al., 2022, Li et al., 2023 and
389 Zhou et al., 2020). Specifically, *SmCry1*, *SmCry2*, *SmCry3* and *SmUVR8* are involved as photoreceptors
390 in response to light. Meanwhile *SmCBF1*, *SmCBF2* and *SmCBF3* have been reported as sensors in
391 response to low temperature. Both sets of genes activate the signaling pathway to produce anthocyanins
392 (Li et al., 2017; Li et al., 2023 and Zhou et al., 2020). Regarding the different downstream stages of the
393 anthocyanin synthesis pathway, there is consensus on the necessary participation of the pathway's
394 structural genes: *SmCHI, SmF3H*, *Sm3GT*, *Sm5GT*, *SmDFR* and *SmANS*) (Jaakola et al., 2013 and Liu
395 et al., 2018. For the intermediate steps, several TFs and their interactions have been proposed in
396 different eggplant populations (R2R3-MYB transcription factor [*SmMYB113*, *SmMYB86*, *SmMYB75*,
397 *SmMYB35*], basic helix–loop–helix [*SmbHLH1*, *SmbHLH13*], *SmHY5*, *SmTT8*, *SmGL3*, *SmTTG1*,
398 *SmCIP7*, *SmGL2*, *SmGATA26*, *SmCOP1*, *SmSPA* and *SmFTSH10*) (Jiang et al., 2016; Li et al., 2017;

399 Li et al., 2018; Qiao et al., 2022; He et al., 2022; Mangino et al., 2022; Zhou et al., 2022; Li et al., 2023;
400 Luo et al., 2023ab and You et al., 2023). Specifically, any mutation in *SmMYB113* or pathway's
401 structural genes generate phenotypes without anthocyanin content (Mangino et al., 2022; Luo et al.,
402 2023ab and You et al., 2023). Therefore, for the PS trait the TFs proposed (*SmHY5*, *SmTT8*, *SmGL3*,
403 *SmTTG1*, *SmCIP7*, *SmGL2*, *SmGATA26*) are in the first steps of the signaling pathway and always
404 upstream of the *SmMYB113* (Mangino et al., 2022, Luo et al., 2023ab and You et al., 2023). In that
405 sense, *SmNAC1-like* agrees with the previous candidate TFs proposed. The homologous of *SmNAC1-*
406 *like* in *Prunus persica* (*PpNAC1*) and *Malus domestica* (*MdNAC1*) are located upstream of the
407 *SmMYB113* homologous in *P. persica* (*PpMYB10.1*) and *M.* domestica (MdMYB10) (Liu et al., 2023
408 and Meng et al., 2023).
409 By using different eggplant materials, we have identified *SmNAC1-like* gene as a promising candidate
410 for the PS trait, with disruptive mutations associated with non-photosensitivity phenotypes. The
411 eggplant PS trait has significant implications for fruit visual quality, as it results in uniform peel
412 pigmentation, which is highly desirable for fruit commercialization. The study of this relevant trait
413 could be of interest for future eggplant breeding projects with the aim of increasing the diversity of the
414 fruit colour palette. In addition, due to a higher concentration of anthocyanin contents and their effect
415 as an antioxidant, *SmNAC1-like* could also be a valuable target for controlling the gene expression of
416 the anthocyanin content in the fruit peel of the eggplants.
417

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CONTRIBUTIONS

JG-C, SM, PG, SV, MP and JP conceived the idea. JG-C and SM supervised and designed the manuscript. JG-C, SM, AA and MDT-T developed the F2 population for BSA-seq and RNA-seq. EG-P developed the introgression lines. JG-C, SM, JL, JS-P, MDT-T performed DNA and RNA extraction. JG-C wrote the manuscript. All the authors reviewed and edited the manuscript.

DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in the NCBI SRA repository, BioProject IDs for BSA-seq are the following: F2 photo-insensitive, SRS26640024, F2 photosensitive SRS26640023, ASI -S-1 SRS26640022 and IVIA-371 SRS26640021. With regards theRNA-

443 se analysis are under the BioProject IDs: PUCR replicate 1 SRR35685429, PUCR replicate 2
444 SRR35685426, PUC replicate 3 SRR35685424, puc replicate 1 SRR35685427, puc replicate 2
445 SRR35685425, puc replicate 3 SRR35685428.

446 **CONFLICT OF INTEREST**

447 The authors declare that the research was conducted in the absence of any commercial or
448 financial relationships that could be construed as a potential conflict of interest.

449 **GENERATIVE AI STATEMENT**

450 The authors declare that no Gen AI was used in the creation if this manuscript or in any of the
451 figure and table attached to the manuscript.

452 **SUPPLEMENTARY MATERIALS**

453 **File S1.** DNA sequence modification in SMEL4.1_10g014730.1 in 67/3 v3, DH-ECAVI and IVIA-
454 371.

455 **File S2.** cDNA and protein sequence of SMEL4.1_10g014730.1 in 67/3 v3 and DH-ECAVI.

456 **Table S1.** RNA quality data of the samples used in the RNA-seq data of PUC and puc.

457 **Table S2.** Evaluation of the gene related with anthocyanin biosynthesis in the QTLs
458 determined by Mangino et al., 2022.

459 **Table S3**. Results of bulk segregation analysis in ASI-S-1 and IVIA-371 with a confidence
460 interval of 95% and 99%, respectively. Eggplant genome reference v4.1

461 **Table S4.** List of genes in the candidate region acotated by BSA-seq in eggplant varieties ASI-
462 S-1 and IVIA-371. Eggplant genome reference v4.1

463 **Table S5.** Results of bulk segregation analysis in ASI-S-1 and IVIA-371 with a confidence
464 interval of 95% and 99%, respectively. Eggplant genome reference v3

465 **Table S6.** List of genes in the candidate region acotated by BSA-seq in eggplant varieties ASI-
466 S-1 and IVIA-371. Eggplant genome reference v3

467 **Table S7.** List of differentially expressed genes in the eggplant varieties IVIA-371 (PUC
468 positive trait) versus ASI-S-1 (puc negative trait).
469 **Table S8.** List of the subcluster identified in the heatmap of the differentially expressed genes
470 of light-independent versus light-dependent eggplant varieties.
471 **Table S9**. List of the enrichment analysis of the GO and KEGG terms of the up- and down-
472 regulated genes in PUC versus puc eggplant fruit peel
473 **Figure S1**. Phenotype of the two sub-introgression lines (IL-69(6)5-12-Ch10 and IL-69(6)5-8-Ch10)
474 derived from the introgression line IL-M5-19.
475 **Figure S2**. Delta of SNP-Index of the bulk segregation analysis of light-dependent and light-
476 independent eggplant varieties
477 **Figure S3**. Number of differentially expressed genes (DEG) in the four subclusters identified
478 in the clustering heatmap PUC vs puc. The DEG in the subcluster 1 and 3 were up-regulated
479 in the light-independent (IVIA-371) eggplant (Panel A and B) meanwhile the DEG in the
480 subcluster 2 and 4 were up-regulated in the light-dependent (ASI-S-1) eggplant.
481

482 **REFERENCES**

483 Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. Available
484 online at: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

485 Aronesty E. 2013. Comparison of sequencing utility programs. The Open Bioinformatics
486 Journal 7, doi: 10.2174/1875036201307010001.

487 Arrones A, Manrique S, Gomis-Cebolla J, Baraja-Fonseca V, Plazas M, Prohens J, Portis E,
488 Barchi L, Giuliano G, Gramazio P, Vilanova S. 2024. Irregular green netting of eggplant fruit
489 peel: a domestication trait controlled by *SmGLK2* with potential for fruit colour diversification,
490 Journal of Experimental Botany, Volume 75, Issue 22, 4. Pages 7066–7078

491 Arrones A, Mangino G, Alonso D, Plazas M, Prohens J, Portis E, Barchi L, Giuliano G,
492 Vilanova S, Gramazio P. 2022. Mutations in the SmAPRR2 transcription factor suppressing
493 chlorophyll pigmentation in the eggplant fruit peel are key drivers of a diversified colour
494 palette. Frontiers in Plant Science 13, 1025951.

495 Barchi L, Pietrella M, Venturini L, et al. 2019. A chromosome-anchored eggplant genome
496 sequence reveals key events in Solanaceae evolution. Scientific Reports 9, 11769.

497 Benjamini Y and Hochberg Y. 1995. Controlling the False Discovery Rate: A Practical and
498 Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society. Vol 57 Issue
499 1 Pages 289-300

500 Cingolani P, Platts A, Wang le L, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM.
501 2012. A program for annotating and predicting the effects of single nucleotide polymorphisms,
502 SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly
503 (Austin) 6(2):80-92. doi: 10.4161/fly.19695.

504 Cabanettes F, Klopp C. (2018) D-GENIES: dot plot large genomes in an interactive, efficient
505 and simple way. PeerJ 6:e4958 https://doi.org/10.7717/peerj.4958

506 Dong NQ, Lin HX. 2021. Contribution of phenylpropanoid metabolism to plant development
507 and plant-environment interactions. J Integr Plant Biol. 63 (1):180-209.
508 Garrison E, Marth G. 2012. Haplotype-based variant detection from short-read
509 sequencing.arXiv preprint arXiv:1207.3907 [q-bio.GN]
510 He Y, Li S, Dong Y, Zhang X, Li D, Liu Y, Chen H. 2022. Fine mapping and characterization
511 of the dominant gene SmFTSH10 conferring non-photosensitivity in eggplant (Solanum
512 melongena L.). Theor Appl Genet. 135(7):2187-2196.
513 Jaakola L. 2013. New insights into the regulation of anthocyanin biosynthesis in fruits. Trends
514 Plant Sci. 18(9):477-83.
515 Jagodzik P, Tajdel-Zielinska M, Ciesla A, Marczak M, Ludwikow A. 2018. Mitogen-Activated
516 Protein Kinase Cascades in Plant Hormone Signaling. Front Plant Sci. 8; 9:1387.
517 Jiang M, Ren L, Lian H et al (2016) Novel insight into the mechanism underlying light-
518 controlled anthocyanin accumulation in eggplant (*Solanum melongena* L.). Plant Sci 249:46–
519 58
520 Junren Meng, Shihang Sun, Ang Li, Lei Pan, Wenyi Duan, Guochao Cui, Juan Xu, Liang Niu,
521 Zhiqiang Wang, Wenfang Zeng. 2023. A NAC transcription factor, PpNAC1, regulates the
522 expression of *PpMYB10.1* to promote anthocyanin biosynthesis in the leaves of peach trees in
523 autumn. *Horticulture Advances*, 2023, 1(1): 8.
524 Lestern-Hasan, S.M.Z. (1991), «Origin and Domestication of the Brinjal Eggplant, Solanum
525 melongena, from S. incanum, in Africa and Asia», en haWKes, J.G. et alii (eds.), Solanaceae
526 III, Taxonomy, Chemistry, Evolution, Londres, Royal Botanic Gardens, pp.369-388
527 Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose program for assigning
528 sequence reads to genomic features. Bioinformatics. 2014 Apr 1;30(7):923-30. doi:
529 10.1093/bioinformatics/btt656.

530 Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows–Wheeler
531 transform. Bioinformatics 25, 1754–1760.
532 Li, H., 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-
533 MEM. arXiv, 1303.3997 [q-bio.GN].
534 Li J, Ren L, Gao Z et al (2017) Combined transcriptomic and proteomic analysis constructs a
535 new model for light-induced anthocyanin biosynthesis in eggplant (*Solanum melongena* L.).
536 Plant Cell Environ 40:3069–3087
537 Li J, He YJ, Zhou L, Liu Y, Jiang M, Ren L, Chen H. Transcriptome profiling of genes related
538 to light-induced anthocyanin biosynthesis in eggplant (Solanum melongena L.) before purple
539 color becomes evident. BMC Genomics. 2018 May 4;19(1):324. doi: 10.1186/s12864-018-
540 4693-y.
541 Li J, Jiang S, Yang G, Xu Y, Li L, Yang F. 2023. RNA-sequencing analysis reveals novel
542 genes involved in the different peel color formation in eggplant. Hortic Res.10 (10):181.
543 Liu M, Li X, Liu Y, Cao B. 2013. Regulation of flavanone 3-hydroxylase gene involved in the
544 flavonoid biosynthesis pathway in response to UV-B radiation and drought stress in the desert
545 plant, Reaumuria soongorica. Plant Physiol Biochem 73:161–167
546 Liu Y, Tikunov Y, Schouten RE, Marcelis LFM, Visser RGF, Bovy A. 2018. Anthocyanin
547 Biosynthesis and Degradation Mechanisms in *Solanaceous* Vegetables: A Review. Front
548 Chem. 6:52.
549 Liu W, Feng Y, Yu S, Fan Z, Li X, Li J, Yin H. 2021. The Flavonoid Biosynthesis Network in
550 Plants. Int J Mol Sci. 22(23):12824.
551 Liu W, Mei Z, Yu L, Gu T, Li Z, Zou Q, Zhang S, Fang H, Wang Y, Zhang Z, Chen X, Wang
552 N. 2023. The ABA-induced NAC transcription factor MdNAC1 interacts with a bZIP-type
553 transcription factor to promote anthocyanin synthesis in red-fleshed apples. Hortic Res
554 10(5):uhad049.

555 Lorenc-Kukuła K, Jafra S, Oszmiański J, Szopa J (2005) Ectopic expression of anthocyanin 5-
556 O-glucosyltransferase in potato tuber causes increased resistance to bacteria. J Agric Food
557 Chem 53:272–281
558 Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-
559 seq data with DESeq2. Genome Biol. 2014;15(12):550. doi: 10.1186/s13059-014-0550-8.
560 Luo L, Molthoff J, Li Q, Liu Y, Luo S, Li N, Xuan S, Wang Y, Shen S, Bovy AG, Zhao J,
561 Chen X. Identification of candidate genes associated with less-photosensitive anthocyanin
562 phenotype using an EMS mutant (*pind*) in eggplant (*Solanum melongena* L.). Front Plant Sci.
563 2023 Dec 11;14:1282661. doi: 10.3389/fpls.2023.1282661. PMID: 38169942; PMCID:
564 PMC10758619.
565 Mangino G, Arrones A, Plazas M, Pook T, Prohens J, Gramazio P, Vilanova S. 2022. Newly
566 Developed MAGIC Population Allows Identification of Strong Associations and Candidate
567 Genes for Anthocyanin Pigmentation in Eggplant. Front Plant Sci. 7; 13:847789.
568 Mattioli R, Francioso A, Mosca L, Silva P.2020. Anthocyanins: A Comprehensive Review of
569 Their Chemical Properties and Health Effects on Cardiovascular and Neurodegenerative
570 Diseases. Molecules. 21; 25(17):3809.
571 Muñoz-Falcón J.E; Prohens J; Vilanova S; Nuez F. (2009). Diversity in commercial varieties
572 and landraces of black eggplants and implications for broadening the breeders' gene pool,
573 154(3), 453–465. doi:10.1111/j.1744-7348.2009.00314.x
574 Moormann J, Heinemann B, Hildebrandt TM. 2022. News about amino acid metabolism in
575 plant-microbe interactions. Trends Biochem Sci. 47(10):839-850.
576 Qiao Jun, Liu Jing, LI Suwen, Wang Liying. 2022. Prediction of Fruit Color Genes Under the
577 Calyx of Eggplant Based on Genome-wide Resequencing in an Extreme Mixing Pool. Acta
578 Horticulturae Sinica, 2022, 49(3): 613-621.

579 Ramsay NA, Glover BJ. 2005. MYB-bHLH-WD40 protein complex and the evolution of
580 cellular diversity. Trends Plant Sci. 10 (2):63-70.
581 Ranil RHG, Niran HML, Plazas M, Fonseka RM, Fonseka HH, Vilanova S, Andújar I,
582 Gramazio P, Fita A, Prohens J. 2015. Improving seed germination of the eggplant rootstock
583 Solanum torvum by testing multiple factors using an orthogonal array design. Scientia
584 Horticulturae 193, 174–181.
585 Ren L, Hu Z, Li Y et al 2014. Heterologous expression of bopap1 in tomato induces stamen
586 specific anthocyanin accumulation and enhances tolerance to a long-term low temperature
587 stress. J Plant Growth Regul 33:757–768
588 Robinson J.T, Thorvaldsdóttir H, Turner D, Mesirov J.P. 2023. igv.js: an embeddable
589 JavaScript implementation of the Integrative Genomics Viewer (IGV). Bioinformatics, 39(1)
590 (2023), btac830.
591 Samuels, J. (2015). Biodiversity of Food Species of the Solanaceae Family: A Preliminary
592 Taxonomic Inventory of Subfamily Solanoideae. *Resources*, *4*(2), 277-322.
593 Sang Z, Zuo J, Wang Q, Fu A, Zheng Y, Ge Y, Qian Z, Cui Y. Determining the Effects of
594 Light on the Fruit Peel Quality of Photosensitive and Nonphotosensitive Eggplant. Plants
595 (Basel). 2022 Aug 12;11(16):2095. doi: 10.3390/plants11162095. PMID: 36015399; PMCID:
596 PMC9416183.
597 Schäfer M, Brütting C, Meza-Canales ID, Großkinsky DK, Vankova R, Baldwin IT, Meldau
598 S. 2015. The role of cis-zeatin-type cytokinins in plant growth regulation and mediating
599 responses to environmental interactions. J Exp Bot. 66(16):4873-84.
600 Speer H, D'Cunha NM, Alexopoulos NI, McKune AJ, Naumovski N. Anthocyanins and
601 Human Health-A Focus on Oxidative Stress, Inflammation and Disease. Antioxidants (Basel).
602 2020 Apr 28;9(5):366. doi: 10.3390/antiox9050366. PMID: 32353990; PMCID:
603 PMC7278778.

604 Taher D, Solberg SØ, Prohens J, Chou YY, Rakha M, Wu TH. World Vegetable Center
605 Eggplant Collection: Origin, Composition, Seed Dissemination and Utilization in Breeding.
606 Front Plant Sci. 2017 Aug 25;8:1484. doi: 10.3389/fpls.2017.01484. PMID: 28970840;
607 PMCID: PMC5609569.
608 Takagi H, Abe A, Yoshida K, et al. 2013. QTL-seq: Rapid mapping of quantitative trait loci in
609 rice by whole genome resequencing of DNA from two bulked populations. The Plant Journal
610 74, 174–183.
611 Takos AM, Jaffé FW, Jacob SR et al (2006) Light-induced expression of a myb gene regulates
612 anthocyanin biosynthesis in red apples. Plant Physiol 142:1216–1232
613 Toledo Garcia, MD. (2024). Regulación genética de la coloración del fruto de berenjena.
614 Universitat Politècnica de València. https://riunet.upv.es/handle/10251/207776
615 Xu S, Hu E, Cai Y, Xie Z, Luo X, Zhan L, Tang W, Wang Q, Liu B, Wang R, Xie W, Wu T,
616 Xie L, Yu G. Using clusterProfiler to characterize multiomics data. Nat Protoc. 2024
617 Nov;19(11):3292-3320. doi: 10.1038/s41596-024-01020-z.
618 Vogt T. Phenylpropanoid biosynthesis. Mol Plant. 2010 Jan;3(1):2-20. doi:
619 10.1093/mp/ssp106. Epub 2009 Dec 24. PMID: 20035037.
620 Wang L, Li J, Zhao J, He C. Evolutionary developmental genetics of fruit morphological
621 variation within the Solanaceae. Front Plant Sci. 2015 Apr 13;6:248. doi:
622 10.3389/fpls.2015.00248. PMID: 25918515; PMCID: PMC4394660.
623 Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, Feng T, Zhou L, Tang W, Zhan L, Fu X, Liu S, Bo
624 X, Yu G. clusterProfiler 4.0: A universal enrichment tool for interpreting omics data.
625 Innovation (Camb). 2021 Jul 1;2(3):100141. doi: 10.1016/j.xinn.2021.100141.
626 Yan H, Pei X, Zhang H, Li X, Zhang X, Zhao M, Chiang VL, Sederoff RR, Zhao X. 2021
627 MYB-Mediated Regulation of Anthocyanin Biosynthesis. Int J Mol Sci. 18;22(6):3103.

You Q, Li H, Wu J, Li T, Wang Y, Sun G, Li Z, Sun B. 2022. Mapping and validation of the epistatic *D* and *P* genes controlling anthocyanin biosynthesis in the peel of eggplant (*Solanum melongena* L.) fruit. Hortic Res. 2;10(2)

Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012 May;16(5):284-7. doi: 10.1089/omi.2011.0118.

Zhang Ying, Zhao Yueqi, Chen Yuhui, Lian Yong, and Liu Fuzhong *. Progress in Molecular Research on Purple Formation of Eggplant[J]. ACTA HORTICULTURAE SINICA, 2019, 46(9): 1779-1796.

Zhang Y, Park C, Bennett C, Thornton M, Kim D. Rapid and accurate alignment of nucleotide conversion sequencing reads with HISAT-3N. Genome Res. 2021 Jul;31(7):1290-1295. doi: 10.1101/gr.275193.120.

Zhou X, Liu S, Yang Y, Liu J, Zhuang Y. 2022. Integrated Metabolome and Transcriptome Analysis Reveals a Regulatory Network of Fruit Peel Pigmentation in Eggplant (*Solanum melongena* L.). Int J Mol Sci. 23(21):13475.

Zhou L, He Y, Li J, Liu Y, Chen H. 2020. CBFs Function in Anthocyanin Biosynthesis by Interacting with MYB113 in Eggplant (Solanum melongena L.). Plant Cell Physiol. 61(2):416-426. doi: 10.1093/pcp/pcz209. PMID: 31725150

646 **TABLES**

647 **Table 1**. Genetic analysis of the segregation of photosensitivity in the F2 population from the
648 cross of ASI-S-1 x IVIA-371. For photosensitivity, only the plants yielding fruits with
649 anthocyanic pigmentation were used.

Cross	Population	Photosensitivity		Total	Expected segregation	Chi-square	p value
		Presence	Absence				
[ASI-S-1xIVIA371] self-crossed	F2	76	18	94	3:1	1.58	0.20

650

651 **Table 2**. QTLs related with anthocyanin biosynthesis (fruit anthocyanin and purple under calyx traits) in different eggplant materials used in the

652 current research and in Mangino et al. (2022), which used as parents of a MAGIC population some of the accessions used in this study.

Type of material	Trait	Accession	Genome version	Chromosome	QTL anthocyanin pigmentation (Mb)		Size (Mb)	Reported
					Start	End		
Introgression Line	Purple under calyx and fruit anthocyanin	IL-69(6)5-12-Ch10	v3	10	84.38*	102.79*	18.41	Current study
Introgression Line	Purple under calyx and fruit anthocyanin	IL-69(6)5-8-Ch10	v3	10	105.85	106.64	0.79	Current study
MAGIC population	Fruit anthocyanin	S3MEGGIC population	v3	1	5.11	5.88	0.77	Mangino et al., 2022
				9	16.2	17.4	1.20	
				10	91.08*	94.81*	3.73	
	Purple under calyx	S3MEGGIC population	v3	1	5.11	5.88	0.77	
				3	7.22	8.65	1.43	
				10	31.54	40.09	8.55	
					2.08	2.67	0.59	
					3.94	4.94	1.00	
					91.08*	94.81*	3.73	
					98.25*	100.55*	2.30	
F2 ASI-S-1 x IVIA-371	Purple under calyx	ASI-S-1	v3	10	84.1*	87.9*	3.80	Current study
		IVIA-371			83.5*	97.7*	14.20	

653 * The QTLs of the IL-69(6)5-12-Ch10, S3MEGGIC population for fruit anthocyanin and Purple under calyx overlap with the candidate regions obtained by

654 BSA-seq in the eggplant accessions IVIA-371 and ASI-S-1 as a reference.

655

656 **Table 3**. List of differentially expressed genes in the QTL of chromosome 10 delimited by bulk segregation analysis in the eggplant varieties

657 IVIA-371 (photoinsensitive) and ASI-S-1 (photosensitive).

Regulation	RNA-seq data			Genome version	Gene description
	Gene Name	log2FoldChange	p value		
UP	*SMEL4.1_10g016630.1*	577,158,472,269,854	6.03E-09	v4.1	CHI-like. Chalcone flavonone isomerase of Nicotiana tabacum
DOWN	*SMEL4.1_10g014730.1*	-8,997,230,728,622	1.40E+04	v4.1	NAC1-like. NAC domain-containing protein 1 of Solanum lycopersicum
	SMEL4.1_10g013610.1	-231,064,107,425,366	7.57E+09	v4.1	Protein of unknown function
	SMEL4.1_10g014530.1	-128,031,116,712,945	0.00011	v4.1	RAD5B-like. DNA repair protein RAD5B of Arabidopsis thaliana
	SMEL4.1_10g019230.1	-114,443,348,682,244	0.00031	v4.1	Protein of unknown function

658

659 **Table 4.** DNA high-impact sequence variants present in the differentially expressed genes located in the QTL of chromosome 10 delimited by

660 bulk segregation analysis in the photoinsensitive and photosensitive eggplant varieties used in this study

Gene Name*	Gene position†		PUC			*puc*	
	Start	**End**	**DH ECAVI**	**IVIA-371**	**AN-S-26**	**H15**	**ASI-S-1**
SMEL4.1_10g016630.1 (SMEL_010g353630.1)	96300106	96301916	-	-	-	-	-
SMEL4.1_10g014730.1 ***(SMEL_010g350010.1)***	88316013	88317850	Position: 88,316,597-602 Reference: AAAGGT Alternate: AGATCGGAAGAGCGTCGT Type: INDEL; Disruptive inframe insertion	Position: 88,317,680-699 Reference: ATATTATTATTATTATTATC Alternate: ATATTATTATTATTATC Type: INDEL; 3_prime_UTR_variant	-	-	-
SMEL4.1_10g013610.1 (SMEL_010g349620.1)	87113226	87117639	-	-	-	-	-
SMEL4.1_10g014530.1 (SMEL_010g349810.1)	88067120	88069892	-	-	-	Position: 88,069,753-756 Reference: TAGG Alternate: AGATCGGAAGAGC Type: INDEL; Conservative_inframe_insertion	-
SMEL4.1_10g019230.1 (SMEL_010g351900.1)	92381275	92381400	-	-	-	-	-

661 *The gene names between brackets belong to the genome version v3 of *Solanum melongena* from Eggplant Sequencing Consortium

662 † The gene position of the DEG located in the QTL of chromosome 10 belong to the genome version v3 of *Solanum melongena* from Eggplant

663 Sequencing Consortium

FIGURES

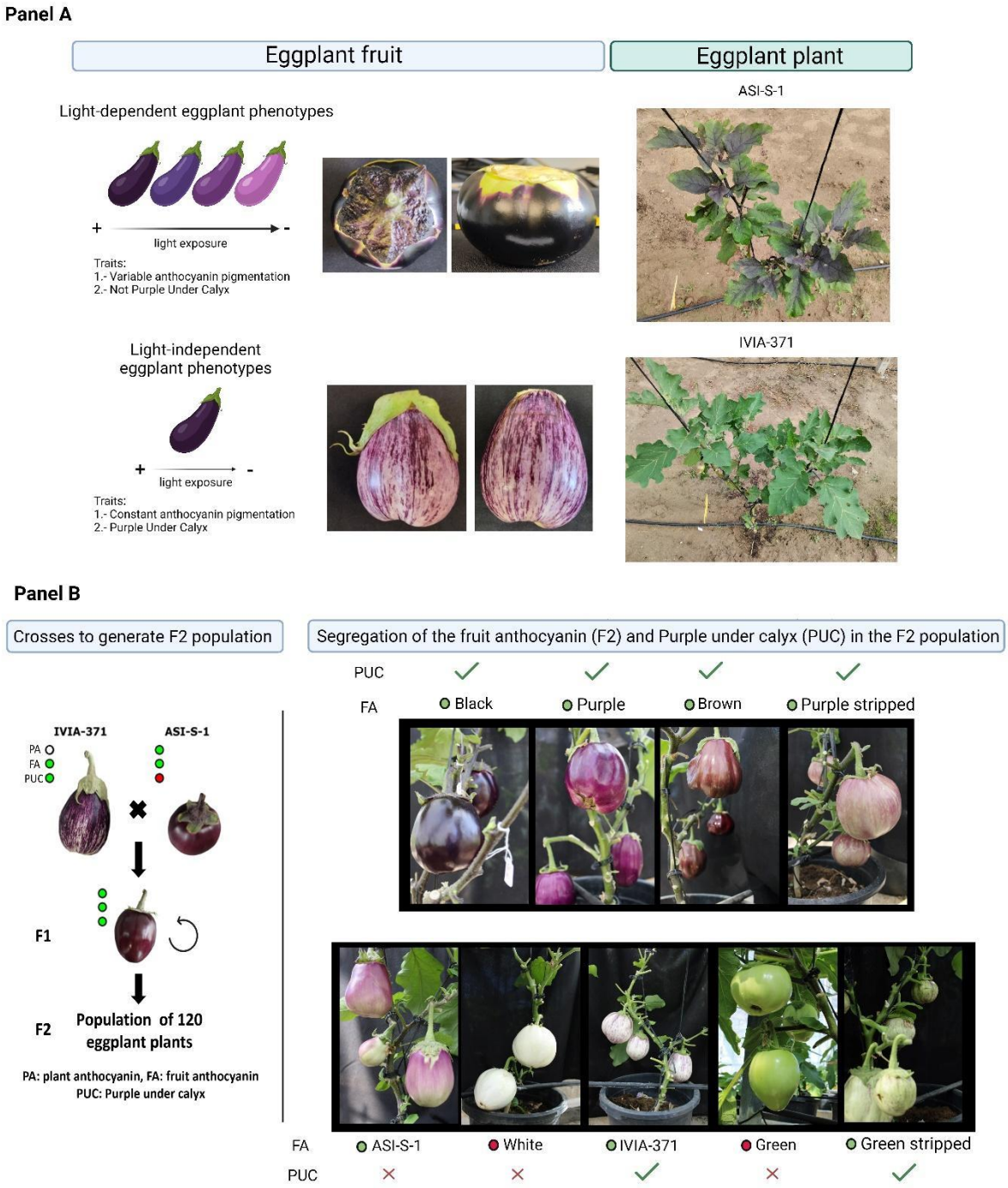


Figure 1. Phenotype of the *Solanum melongena* varieties photosensitive (ASI-S-1) and photoinsensitive (IVIA-371) and F2 population. Panel A: Phenotype of the eggplant fruit of ASI-S-1 and IVIA-371. Panel B: Crosses performed to develop the F2 population and the different F2 phenotypes obtained for fruit anthocyanin (FA), purple under calyx (PUC) and chlorophyll content (CC).

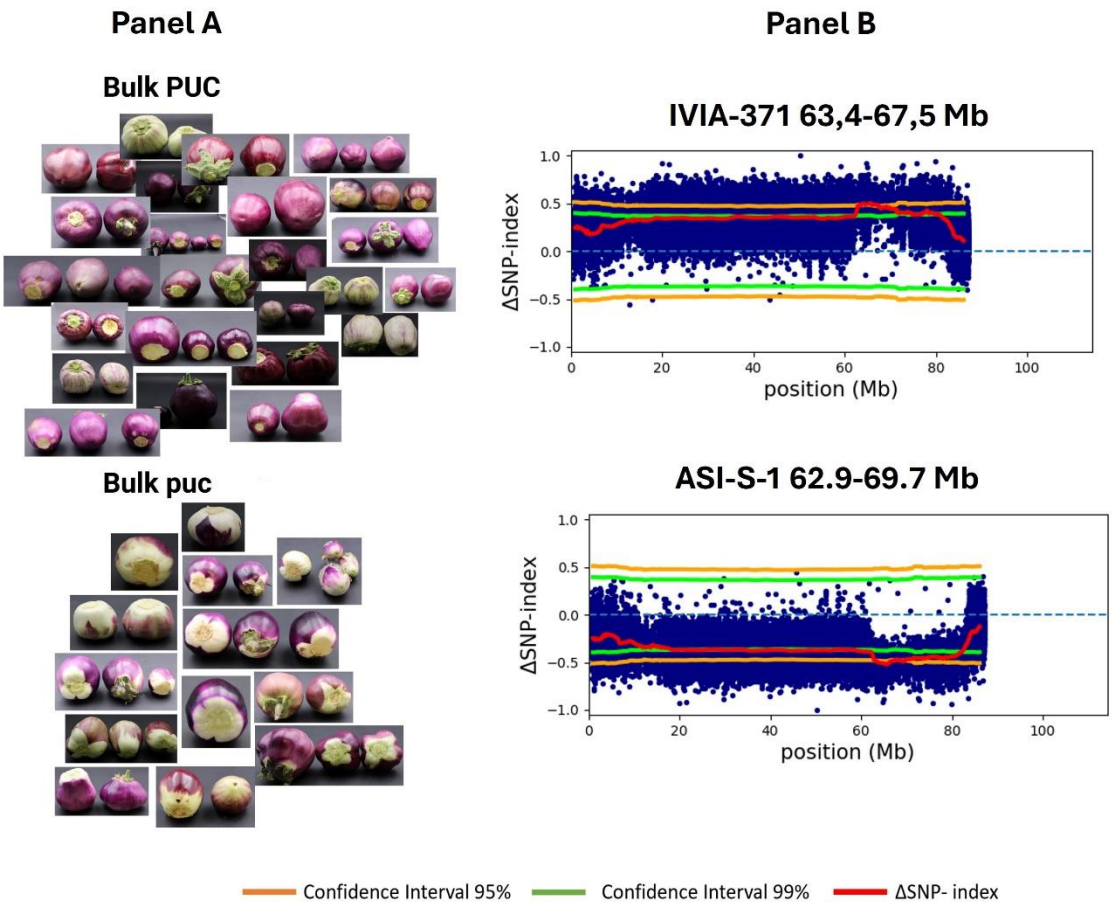
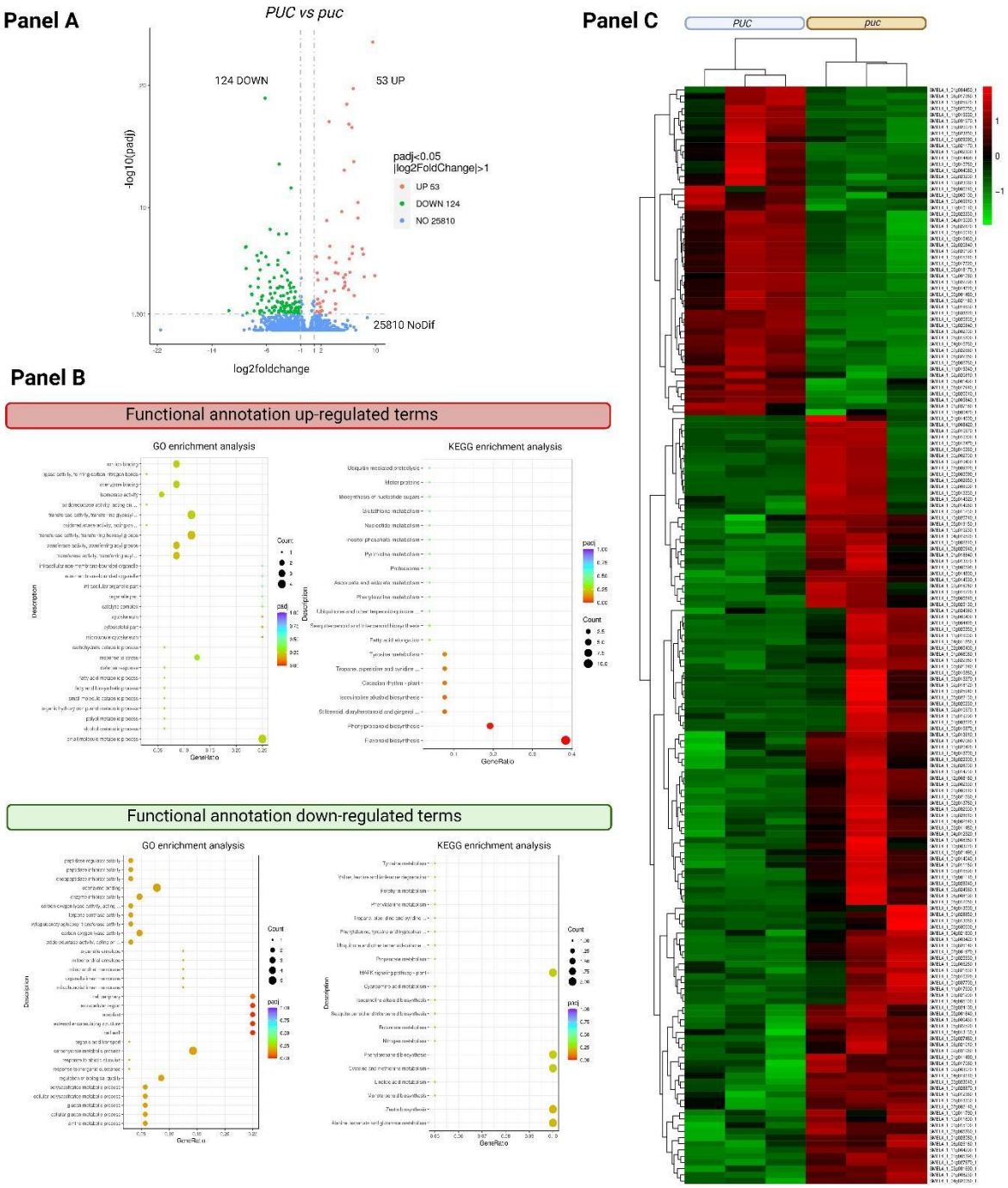


Figure 2. Results of the bulk segregation analysis in the F2 population of IVIA-371 (photoinsensitive) x ASI-S-1 (photosensitive). Panel A: Phenotype of the eggplants used to generate the bulks. Panel B: ΔSNP-index and the position of the respective QTL for IVIA-371 and ASI-S-1, respectively.



676

677 **Figure 3**. Differentially expressed genes (DEG) in early bagged photoinsensitive (IVIA-371)
678 versus photosensitive (ASI-S-1) eggplants. Panel A: Vulcano plot of the up- and down-
679 regulated of the fruit peel in early developed bagged eggplants. Panel B: Enrichment analysis
680 of the up- down-regulated terms in GO and KEGG terms. Panel C: Clustering heatmap among
681 the PUC vs puc and the DEG expressed in the eggplant fruit peel.