Brief Communication

CRISPR/Cas9-mediated gene editing of GmJAGGED1 increased yield in the low-latitude soybean variety Huachun 6

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The number of seeds per pod (NSPP) is one of the critical components that affect soybean yield (Liu et al., 2020). In, which encodes GmJAGGED1 (GmJAG1), is a major locus regulating NSPP and leaflet shape in soybean (Fang et al., 2013; Jeong et al., 2012). A single base pair substitution of the In locus from guanine (G) to cytosine (C) led to an amino acid change in the conserved EAR motif of GmJAG1, resulting in the loss of function of GmJAG1 and In (Fang et al., 2013; Jeong et al., 2012). In can significantly increase NSPP (Fang et al., 2013) and has been widely used in soybean breeding. By introducing In into Kedou 1, the yield was increased by approximately 8% to 10% and resulted in a new high-yield variety Kedou 17 (Liu et al., 2020).

In order to breed the superior varieties for soybean in the tropical and subtropical regions, we intend to fulfil the advantage of the beneficial In locus. For that purpose, we analysed the distribution of the natural In allele in soybean populations. Among the total of more than 3800 accessions that have been re-sequenced (Fang et al., 2017; Torkamaneh et al., 2021; Zhou et al., 2015), In widely exists in the modern elite lines and less frequently exists in landraces (Fig. 1a). Interestingly, In was hardly found in wild soybean (Glycine soja). The nucleotide diversity around the In locus was extremely low, in strong contrast to Ln (Fig. 1b,c). This result is consistent with the previous report that In is located in a selection sweep (Fang et al., 2013) and indicates the In allele has been favourably selected for the modern soybean breeding practices, which leads to an enrichment of In in modern soybean lines.

Among the 173 re-sequenced accessions collected from south and Southeast Asia and Brazil (Fang et al., 2017; Torkamaneh et al., 2021; Zhou et al., 2015), we did not find In. In order to find In from the low-latitude soybean varieties, we collected an additional 210 accessions from south China and sequenced GmJAG1 by Sanger sequencing. However, In was still not found, indicating that In does not exist in the soybean germplasms that originated from low latitudes (Fig. 1a). Soybean is hypersensitive to photoperiod, which limits the intercross breeding between the varieties from different latitudes. Thus, it would be highly challenging to create In-carrying varieties in low latitudes by intercrossing between In from high latitudes and varieties from low latitudes.

Given the nature that In is the mutation of GmJAG1, it would be feasible to introduce the In-related beneficial traits into the varieties of low latitudes by mutating GmJAG1. For that, we employed the CRISPR/Cas9-based genome-editing technology and edited GmJAG1 in a low-latitude variety Huachun 6, which is a spring soybean cultivar and has been certified by the Chinese government (Fig. 1d). Two guide RNAs were designed to target GmJAG1 (Fig. 1d). We obtained two independent T0 lines carrying homozygous mutations of GmJAG1. Due to the high sequence similarity between GmJAG1 and GmJAG2, GmJAG2 was also edited (Fig. 1d). From the T1 segregation population, we identified a CAS9-free homozygous mutant, which was named as gmjag. Sanger sequencing showed that the start codons of GmJAG1 and GmJAG2 were lost in gmjag, in addition to the small deletions on the second exons of both genes (Fig. 1d). This plant and its offspring were used for further trait analysis. Consistent with the varieties carrying In, gmjag showed narrow leaflets (Fig. 1e).

To evaluate the performance of gmjag, we conducted two field trials at Guangzhou experimental stations (N23°15′, E113°34′) in the summer of 2020 and the spring of 2021, respectively. The growth period, plant height, number of branches, number of nodes and number of total pods were not changed in gmjag compared with Huachun 6. Interestingly, we observed 4-seeded pods in gmjag plants, whereas Huachun 6 did not develop any 4-seeded pods (Fig. 1e). Additionally, the percentage of 3-seeded pods was significantly increased in gmjag, whereas that of 2-seeded pods was significantly decreased (Fig. 1f,g). The average NSPP and total seeds per plant of gmjag were significantly increased compared with Huachun 6. Although the single seed weight of gmjag was lower than that of Huachun 6 in the summer trial, no significant difference was observed in the spring trial (Fig. 1h). The yield of gmjag was increased by 8.81% and 8.67% in the spring and the summer trial, respectively. These results indicate that CRISPR/Cas9 knockout of GmJAG1 is effective in introducing In-related beneficial traits into the varieties in low latitudes.

CRISPR/Cas9 technology has been widely proposed as a powerful tool for rapid crop improvement. This work not only created new superior soybean germplasm by CRISPR/Cas9-mediated gene editing but also provided a template for utilization of CRISPR/Cas9 technology in legume crop breeding.
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Conflict of interest
The authors declare no conflict of interest.

Author contributions
HN and LG designed the research; ZC, PX, YC, QM and TL performed the experiments; ZC and PX performed data analyses and ZC and LG wrote the manuscript.

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