

OIL REGULATORY GENES OF BRASSICA JUNCEA (INDIAN MUSTARD): A REVIEW**CHANDAN BHARTI MISHRA****Abstract**

Improvement in seed oil content is a major target of plant breeders and biotechnologists worldwide. Import of edible oil in our own country to meet our demand-supply gap cost several thousands of crores in valuable foreign exchange. Therefore development of strategies to increase oil content of oilseed crops is of paramount importance. Brassica species comprise oilseed and a variety of vegetable crops. In the Indian subcontinent, Brassica juncea (Indian mustard) is the major oilseed crop after groundnut. Oil is mostly accumulated in seeds in the form of Triacylglycerols (TAGs). These TAGs are synthesized from fatty acyl-CoA and glycerol-3-phosphate in the endoplasmic reticulum of the cell. Fatty acids are exclusively synthesized in the plastids from acetyl-CoA and then transported in the form of fatty acyl-CoA to the cytoplasm. TAGs are synthesized by the stepwise acylation of glycerol-3-phosphate in the endoplasmic reticulum, (Kennedy pathway). Phosphatidic acid is formed from glycerol-3-phosphate with the help of two enzymes, glycerol-3-phosphate acyltransferase and lyso-phosphatidic acid acyltransferase, which add fatty acyl moieties to the sn-1 and sn-2 positions of glycerol-3-phosphate respectively. Phosphatidic acid is hydrolysed by the enzyme phosphatidate phosphatase to give diacylglycerol (DAG). TAGs are synthesized by the addition of a third acyl chain to DAG by the enzyme diacylglycerol acyltransferase.

Key words: Brassica juncea, GL2, DAG, TAG

Introduction

Brassicajuncea, Indian oilseed mustard, is an important oilseed crop of the country. This crop is grown in the north-western region of India in around six million hectares of land during the winter growing season either under completely rainfed or with limited irrigation. The average yields of the best check varieties in India are around 2.2 tonnes per hectare. Therefore even minor improvement in the quality and yield of this crop would markedly improve the income of our farming community. B. juncea (AABB genome) is an allopolyploid species produced by crosses between two diploid species viz. B. rapa (AA genome) and B. nigra (BB genome). The existence of two distinct and genetically diverse gene pools namely, the east European and the Indian gene pools in B. juncea, has been reported earlier^[1] had shown that the lines belonging to the Indian gene pool show B. rapa (AA) like characters whereas those belonging to the east European gene pool show closeness to B. nigra (BB) for many agronomic and yield influencing traits. However, the east European lines, which are generally tall and late in maturity, are poor yielders as they are ill adapted to winter growing conditions of the Indian subcontinent.

Recent studies on QTL mapping of oil content under zero-erucic conditions in *B. juncea*, identified two novel loci on linkage groups A2 and A3 that explain ~19% phenotypic variance for oil content. Using a syntenous map developed between the model plant, *Arabidopsis*, and *B. juncea*^[3] comparative analysis of the above QTL regions had identified two potential candidate genes for oil content *WRI1* and *GLABRA2*^[2].

Extensive studies done previously in *Arabidopsis* showed that the *GL2* gene is involved in the development of normal trichomes, atrichoblasts, seed coat epidermal cells and limits hair formation in roots in addition to controlling seed oil content^{[3][4][5][6]}. Studies on *gl2* mutant plants showed deviation from the normal expansion of trichomes. Most trichomes found on the mutant plant were either enlarged abortive epidermal cells that expanded in the plane of the leaves or they were un-branched spikes. The trichome wall did not appear to be thickened and neither did they acquire papillae^[5].

A number of genes upstream to *GL2* gene have been identified that positively regulate the expression of *GL2* thus controlling trichome and root hair development. For normal trichome initiation, a myb-type transcription factor encoded by *GLABRA1* (*GL1*) gene and a WD40 protein encoded by *TRANSPARENT TESTA GLABRA 1* (*TTG1*) gene are required. Complete loss of leaf trichome initiation resulted due to loss of function mutations in *gl1* and *ttg1* mutants of *Arabidopsis*^[7]. The double mutants, *gl2 gl1* and *gl2 ttg* also lack trichomes showing that the *GL2* gene acts downstream to *GL1* and *TTG* genes. Further, the level of *GL2* expression in *ttg* roots was reduced, suggesting that the *TTG* gene plays a role in up-regulating *GL2* expression^[8]. Similarly *GLABRA 3* (*GL3*) and *ENHANCER OF GLABRA 3* (*EGL3*) are two homologous genes encoding bHLH transcription factors and *gl3/egl3* double mutants showed a complete loss of trichome development^[5].

In addition to the above, *GL2* is also required in the development of seed coat epidermis, and seed coat mucilage biosynthesis. The latter role is executed through positive control of the *MUCILAGE MODIFIED 4* (*MUM4/RHM2*) gene^[9], which encodes the rhamnose synthase enzyme required to produce rhamnose, a key substrate for mucilage biosynthesis. Recent studies showed that mucilage deficient *gl2* seeds of *Arabidopsis* produce more oil and *MUM4* is a downstream target of *GL2* gene. *MUM4* activity along with *GL2* is required for normal seed oil accumulation in the seed coat. However, loss of function of *MUM4* resulted in mucilage deficiency leading to higher seed oil content. The phenotype of *gl2/mum4* double mutant was similar to that of both parents suggesting that both *GL2* and *MUM4* function in the same pathway influencing seed oil biosynthesis. These studies strengthen the concept that *GL2* negatively regulates seed oil content by positively regulating *MUM4*. However it is still not clear whether *GL2* directly targets and regulates *MUM4* or other downstream intermediate genes are also involved in seed oil biosynthesis. Shi et al. (2012)

studied the seed oil content of various mutants like TTG1, TTG2, MYB5, TT2, EGL3, TT8, GL2 and MUM4, which were disrupted in components of the regulatory pathway for mucilage production. Their results showed that the oil content of *ttg2* seeds was comparable to that of the wild type, suggesting that it is not just the deficiency or loss of mucilage that results in over production of seed oil but in fact, is the loss of MUM4 function that is responsible for higher seed oil content.

The GL2 gene encodes a homeodomain (HD) transcription factor, belonging to the class IV homeodomainleucine zipper (HD-ZIP) gene family, based on its protein sequence^[4]. Homeodomain proteins are transcription factors which function during diverse developmental processes, to coordinate the expression of several target genes (reviewed by Kessel and Gruss, 1990; Gehring et al., 1994). At least fifteen homeodomain proteins are known to be present in Arabidopsis^[4]. The HD-Zip group, to which GL2 genes most closely related, contains towards its N terminus, a homeodomain which is then followed by an amphipathic alpha helical domain ^[10]. Under in vitro conditions, the alpha helical region of GL2 is capable of interacting with itself. GL2 encodes an additional 527 amino acid residues towards the C-terminal of the helical domain. Although this region does not contain any recognizable motifs, other genes that show significant similarity to the C-terminal domain of GL2 have been identified. For example, GL2 and Arabidopsis Thaliana Meristem Layer 1 (ATML1) genes are 37% identical throughout their sequence but 60% identical in the homeodomain^[11]. GL2 also shares C-terminal sequence similarity to homeodomain genes from Phalenopsis, Helianthus and Panicum.

Conclusion

The expression of GL2 gene in each cell type is activated by a transcription complex which includes polypeptides from three classes of transcriptional regulators. A WRKY transcription factor TRANSPARENT TESTA GLABRA 1 (TTG1), a basic helix-loop-helix (bHLH) transcription factor and a MYB transcription factor^{[9][11]}.

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