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BIOCHEMICAL COMPOSITION OF GM FLAX AND IT'S RELATION TO PLANT PRODUCTIVITY

ABSTRACT

Flax (Linum *usitatissimmum L.)* is one of the valuable crops that are used for different applications. The name of flax family, *Linaceae* recognizes its utility for a variety of applications. Flax is considered as non-waste plant as each part of it has different uses. Flax seeds are used as food, and it is oil have both nutritional and industrial applications. The flax seedcake is rich in phenolic compounds that have potential medicinal properties. Flax fiber is applied in the textile industry, and nowadays it is used in biocomposites and biofuel production. Even flax shives are used for biofuel production and could be used as a source of valuable components.

Flax fiber varieties cultivation decreased in the last 40 years for many reasons. Some of those reasons were political and economical. Other reasons were correlating with flax fiber properties. One of the primary reasons was flax susceptibility to pathogen infection. Fusarium oxysporum that causes *Fusarium* wilt is classified as the most dangerous pathogen for flax. It could reduce the quality and the quantity of the flax yield .

Genetic engineering was used to improve the mechanical properties of flax fiber. Two primary methods were used to improve the mechanical properties of flax fiber. The first method was by silencing cinnamyl alcohol dehydrogenase (CAD) enzyme to reduce lignin content. Reducing lignin content in CAD plant has improved the fiber quality by improving the fiber elasticity. The second method that was used to improve fiber quality was by reducing pectin content. The pectin reduction was due to the overexpression of polygalacturonases. Decreasing pectin content was to shorten the retting time. PGI 11 plant has also shown better mechanical properties as compared to the control plant. However, changes in the transgenic lines ressitance to pathogen infection were observed in the *in-vitro* lines. The plant productivity is affected by many factors. One of the main factors affecting it is plant resistance or susceptibility to pathogen infection.

The aim of this study was to determine the changes in the biochemical composition of the biopolymers in flax seed and flax plant of the

transgenic lines, caused by the introduced modifications, to determine factors that correlate with the observed changes in the transgenic lines resistance to *Fusarium* infection. In this study, three stages of plant development were investigated: seeds, 4-week old and 11-week old plant. The flax seeds contain all the material which plant used during germination and early stages of emergence. At 4-week old, the reserves of the seed biopolymers are almost finished and the plant depends on what it have built from photosynthesis in it is development and ressitance against pathogen infection. At 11 week old, the plant is almost mature, and any infection could cause significant losses in the yield of the crop.

To detrmine the transgenic lines resistance to pathogen, the seed were germinated on MS medium, and after the seedlings grew, the media were infected by *F. oxysporum* or *F. culmorum*. The seedlings resistance against *Fusarium* infection was then investigated. PGI 11 seedlings were more resistant compared to control, while CAD 33 plants were more susceptible to *Fusarium* infection. Moreover, we investigated the seed vitality by emergence test. The seed vitality was decreased in CAD 33 while it was improved in PGI 11 plant.

To investigate the changes in the first developmental stage, seeds, we have studied their biochemical composition. The main biopolymers content was determined: cellulose, hemicellulose, lignin, and pectin. The sugar composition of pectin and hemicellulose was analyzed by ultraperformance liquid chromatography (UPLC), as well as mucilage content and composition . The phenolic compounds content was also analyzed by UPLC. In addition, fatty acid composition was determined by gas chromatography (GC) with FID detector.

The effect of the modification was pronounced in seeds. Lignin content was decreased dramatically in CAD 33 seeds while pectin content was reduced significantly in PGI 11 seeds. In addition, other biopolymers were also affected by the modification. For example, both pectin and hemicellulose contents were increased in CAD 33 seeds while cellulose content was decreased significantly. Moreover, lignin content was decreased in PGI 11 plant while hemicellulose content was increased. Phenolic compound content was decreased significantly in CAD 33 plant and slightly reduced in PGI 11 plant. No significant changes were observed in the fatty acid composition of the transgenic lines as compared to the control plant.

In 4 and 11-week old plant, we investigated the biochemical composition of the major biopolymers of the flax plant. As in seeds the main biopolymers, cellulose, lignin, pectin and hemicellulose content was determined. In addition, the sugar composition of the pectin and hemicellulose was analyzed by UPLC, as the content and composition of phenolic compounds. Furthermore, the gene expression of the metabolism genes that participate in the biosynthesis of these biopolymers was studied. The gene expression was analyzed using Real-time PCR method. Moreover, the pathogen-related (PR) gene expression of β1, 3glucanase, and chitinase was analyzed.

However, the 35S promoter was used in the modification; the changes in the biopolymers were not pronounced as in seeds. No reduction in the lignin content was observed in CAD 33 plant, even it was slightly increased. The reduction in pectin content in PGI 11 plant was slight and not significant as it was in seeds. As in seeds, both pectin, and hemicellulose content increased in CAD33 plant. Further, in PGI 11 plant, lignin content was decreased while hemicellulose content was increased. However, as in seeds, phenolic compound content was decreased significantly in CAD 33 while a slight reduction was observed in PGI 11 plant.

The gene expression result explains some of the biochemical composition outcomes. In CAD 33 plant, however, CAD gene was silenced, the overexpression of sinapyl alcohol dehydrogenase (SAD) gene has compensated the reduction in CAD. The PGI 11 plants have an overexpression of β1; 3glucanase, and this could indicate that the plant is more ressitance to pathogen infection. In addition, in CAD 33 plant both PR genes expression, β1,3glucanase, and chitinase, were repressed significantly.

In the third stage, 11-week old plant, the modifications were pronounced. The lignin content was reduced significantly in CAD 33 plant. Further, pectin content was not changed as compared to the control plant. In addition, cellulose and hemicellulose content were decreased in CAD 33 plant. In PGI 11 plant; pectin content was decreased significantly. Both cellulose and lignin content were increased significantly in PGI 11 plant. A dramatic reduction in hemicellulose content was observed in PGI 11 plant. As in seeds and 4-week old plant, phenolic compound content was reduced in CAD 33 plant insignificantly and slightly in PGI 11 plant. The gene expression result supports the biochemical analysis. Both CAD and SAD gene expression was decreased in CAD 33 plant. The reduction in hemicellulose content in PGI 11 plant is a result of the overexpression of 1,4-alpha-xylosidase (XYLa) gene that responsible for hemicellulose

degradation, especially the significant decrease in xylose content. In addition, chitinase gene was overexpressed in PGI 11 plant while it was repressed in CAD 33 plant.

Indeed changing the cell wall composition in transgenic seeds affects the plant resistance in the early stages of plant life. The oligosaccharides, which are released from the homogalacturonan chains in pectien due to introducion of the polygalacturonase in PGI 11 plant seem to work as elicitors that induce the defense system in plant against pathogen. This state of induced ressitance was responsiable for the overexpression of the PR genes and WAK gene that improve the plant ressitance. In addtion, the physical barriers were developed, especially in 11-week old plant where cellulose and lignin content was increased. The productivity was improved in PGI 11 plant due to the introduced modification.

On the other hand, silencing CAD gene had negative effect on plant ressitance in CAD 33 plant. The plant reacts as there was not any signal of pathogen attack. This state was responsiable for the reduction in expression of PR genes, phenolic compound content and the antioxidant activity of CAD 33 plant and as a consequance increased the plant susceptabilty to pathogen infection.

Reducing pectin content by introducing fungal polygalacturonase was therefore a better stratgy to improve fiber properties , plant resistance and overall the plant productivity compared to reducing lignin level by silencing CAD gene.