'Green Revolution' Gene(s) Related Stem Morphogenesis in Mulberry (*Morus* spp.)

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ABSTRACT

Gibberellins (GAs) are classic plant growth regulator group that has various functions in plant organs development and growth. GAs were frequently reported controlling stem or internodes elongation in vegetative stage as well as inducing inflorescence growth at generative stage. The presence of GAs in plant cell is responded by GA signaling protein. GAI and other members of DELLA protein play an important role of GA signaling in plants. DELLA protein family genes are widely known as 'green revolution' genes, since members of these family genes were found to be the key for semi dwarf character development in cereals which brought to the world production increased during 1960th to 1970th. Mulberry trees (Morus spp.) are dicotyledonous plants that widely use in sericulture activities for silkworm feed. As plants that leaves will be the target for utilization, stem growth and development of mulberry trees is important trait to be studied and characterized. This characterization might be useful for improvement of plant stature and architecture of the mulberry trees that may reduce unnecessary labor cost and maintenance for shoot topping in mulberry cultivation. Herein, study and characterization of the bending stem character of mulberry 'Garyu' which is a mulberry genetic resource in Japan were performed. The effects of GA and abscisic acid (ABA) on mulberry growth were examined compared with the normal straight stem mulberry genotypes. Gibberellin response modulator genes, GAI-like, were cloned from mulberry and sequenced to elucidate the mutation domain of gene sequence, which may relate to the development of mulberry 'Garyu'. Hormonal system was previously hypotesized to be the control of the morphological bending stem development and growth in mulberry 'Garyu'. This hypotesis came from the indication that mulberry 'Garyu' has shorter internodes length comparing to normal straight stem type of mulberry. GAs are widely known to be the plant growth regulator or plant hormones that control of stem elongation growth in higher plants, whereas the ABA is known to be in the contrary to gibberellins function, it is mainly repressed the plant growth especially in environmentally stress condition. The gibberellin's GA_3 type, natural type of abscisic acid ((S)-(+)-ABA) and their mixture were tested by applying them to the mulberry 'Garyu' which bending stem type and other normal straight stem types of mulberry. It was found that the mulberry 'Garyu' did not respond to the application both vegetatively or generatively, while in mulberry leaves development mutant 'Ryoumenguwa' that exhibit straight stem type responded by showing significant increase in leaves (vegetative) and flowers (generative), and also in mulberry wildtype 'Ichibei' showed significant increase only in flowers (generative). From this phenomenon, further study was performed in molecular level to analyze whether it has relation to the gibberellin insensitive phenomena in plants which control by DELLA protein genes family, the members of GRAS transcription factor genes family. It was then revealed that there are slight differences in the nucleotides sequence of GAI-like gene of mulberry 'Garyu' comparing to the other type of mulberry. Two nucleotides difference were found to be potential of changing the amino acids component in GAI-like protein of mulberry 'Garyu'. The changes region of DELLA protein genes that frequently reported in plants were found at DELLA region and/or HYNP/TVHYNP region such as in Arabidopsis, rice, maize, wheat, grapevine and apple. Mutation of DELLA region or HYNP / TVHYNP region in GAI gene ortholog frequently altered dwarfing growth or disrupting transcription level for GA sensitivity (Peng et al., 1999 ; Ogawa et al., 2000 ; Boss & Thomas, 2002 ;

Hedden, 2003 ; Foster et al., 2007), and in mulberry 'Garyu' one of the potential changes was found in TVHYNP region of its GAI-like gene in this study.