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# Morphological Traits of S598A Sweetpotato as an Industrial Starch Crop

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**ABSTRACT** Sweetpotato is one of the important starch crops, current more considered as an industrial crop rather than food because it has higher starch content (over 80% of biomass), it is used for bio resources for industrial area. In this study, we generated S598A (a mutant gene of oat phytochrome A) sweetpotato plant using *Agrobacterium*-transformation method. Morphological characteristics of S598A plant were compared with the wild type sweetpotato, S598A had darker green leaves, increased chlorophyll content higher than to two-fold, delayed leaf senescence, shorter plant height (60% shorter than that of the wild type), more number of leaves and petioles about 1.8-fold, shorter petiole length (30% shorter), 1.2-fold more branches and 1.6-fold thicker stem diameters. From this study, S598A plants with such phenotypic characteristics might be able to use the solar energy efficiently, to have increased tolerance to biotic and abiotic stresses and finally to increase productivity (not only starch yield but also root biomass yield). S598A sweetpotato lines are under field trials.

*Keywords* : semi-dawfism, morphology, phenotype, photosynthesis, S598S, starch, sweetpotato

**Sweetpotato**[*Ipomoea batata* (Lam) L.] is one of the important crops as food, feed and industrial crop that, because of its strong adaptability to drought, infertile soil condition and limited management practice (Woolfe, 1992), can be grown on marginal lands such as vulnerable to those environmental stress.

These day the usage of sweetpotato is extended to diverse fields such as food, feed, functional food and the replacement of petrochemical industry (biofuel, bioplastic, (http://www.uncapsa.org/Flash/flash0803.pdf) because its carbohydrate content higher than any other starch crops and enough to be used as starch crop for industrial application.

Although dried chopped sweetpotato roots have used in brewing industry, the production of sweetpotato for the industrial purpose has stagnated in recent. In 1965, sweetpotato was cultivated in 152 thousand ha (http://goheung.jares.go.kr/nongjin/A060601.html), but in 2007 its cultivation with 21.1 ha (http://www.knrda.go.kr/ares/market/m106.htm). Its cultivation area in 2007 reduced more than 85% compared with that in 1965.

Plants have evolved mechanisms that enable them to respond to the presence of neighbours. The spectral energy distribution of daylight is dramatically altered by vegetation (Smith & Whitelam, 1997). The photosynthetic pigments absorb light over most of the visible spectrum, although some green light is reflected or transmitted. Radiation in the FR (far red) region is very poorly absorbed and, consequently, the light that is transmitted through, or reflected, from vegetation is depleted in R (red) and significantly enriched in FR wavelengths.

In special, sweetpotato plants compete highly between/among intra- and inter plants with light, higher leaves of the plant absorb enough light and use it for photosynthesis, whereas full sun light cannot be transmitted to the lower leaves, thus, the lower leaves of plants use less light (FR, shadow) for photosynthesis and early meet senescence.

Plants have evolved several different classes of photoreceptors to monitor light quality and quantity. Among them the phytochromes are the most popular and important photoreceptor in plant. The phytochromes exist in two distinct but interconvertible forms, the R light-absorbing Pr form and the FR light-absorbing Pfr form. The Pfr form is generally

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considered to be the biologically active form. The red (R) /far-red (FR) light absorbing phytochromes are the well known character reflects the responses to light environment *in planta*. In *Arabidopsis thaliana*, the five phytochromes (phyA to phyE) are generally categorized into two groups: light labile type (phyA) and light stable type (phyB, phyC, phyD and phyE) (Sharrock & Quail, 1989).

Kim et al (2004) investigated that the functional role of phytochrome phosphorylation in plant light signaling using a Pfr-specific phosphorylation site mutant, Ser598Ala (S598A) of oat (*Avena sativa*) phytochrome A (phyA). The transgenic *Arabidopsis thaliana* (*phyA-201* background) plants with this mutant phyA (S598A) showed hypersensitivity to light compared with the transgenic plants with wild-type oat phyA, suggesting that phytochrome phosphorylation at Serine-598 (Ser598) in the hinge region is involved in an inhibitory mechanism.

Thiele *et al.* (1999) characterized morphologically and physiologically potato (*Solanum tuberosum*) plants expressing *Arabidopsis* phytochrome B to explore their potential for improved photosynthesis and higher tuber yields, and they reported that transgenic plant had higher photosynthetic performance and the longer lifespan allowing greater biomass production, resulting in extended underground organs with increased tuber yields.

Sweetpotato is considered to be important crop as industrial crop. This was performed to investigate the possibility of increased starch yield in sweetpotato by modification of morphological traits, and the changes of morphological traits of sweetpotato plant expressing a mutant oat phyA (called S598A) were measured

## MATERIALS AND METHODS

### Plant material

Sweetpotato embryogenic calli were induced from meristem of a sweetpotato [*I. batata* (L.) Lam] cv. Yulmi and they were maintained according to the method of Shin et al (Shin *et al.* 2007).

### Transformation and production of S598A plants

Transformation: In pCAMBIA2301-S598A, S598A gene was cloned in pCAMBIA2301 (CAMBIA, Australia) backbone which contains NPTII (Neomycin phosphotransferase II)

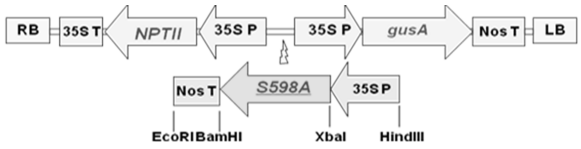



**Fig. 1.** Map of vector pCAMBIA2301-598A. 35S P, CaMV35S promoter; Nos T, Nos polyA; 35S T, CaMV35S poly A, NPTII, neomycin phosphotransferase II; S598A, a oat mutant phytochrome A.

selectable marker controlled by both CaMV35S promoter and terminator, and S598A gene which is oat mutant PHYA was controlled by CaMV35S promoter and NOS terminator. pCAMBIA2301-S598A (Fig. 1) was transferred into *Agrobacterium tumefaciens* strain EHA101. Transformation was performed by the described method of Yi *et al.* (Yi *et al.*, 2007). For the selection of transgenic calli and plants, G418 and paromomycin were used in both *in vitro* and *in planta* (Shin *et al.*, 2007). Primary plants with well-developed roots were transplanted onto the greenhouse and stems from the primary plants were transplants onto 50-cm pots.

PCR and Southern blot analysis. Genomic DNA was isolated from both wild type and S598A plants (Dellaporta *et al.*, 1983). PCR was performed with *Taq* DNA pol (TaKaRa Bio, Japan) in a thermal cycler (Gene Amp® PCR system) (Applied Biosystems, USA). PCR condition described by Shin *et al.* (2007) was followed. The primer sequence designed for 1kbp of S598A was followed as: forward 5'-ACATGG ATGACAGCAGAAGGATGC-3' and reverse 5'-ATGTTG CAGCTCATGACTAGCAAC-3'. For Southern blot analysis, 10μg of each DNA was digested with *Eco*RI in 50μl of the manufacturer's buffer (New England BioLabs, USA) and The DNA was hybridized a 1-kb fragment of S598A. Blotting was followed by the description of the Shin *et al.* (2007).

Measurement of morphological traits. Plants of wild type and S598A sweetpotato plants were transferred to the 50-cm pots containing soil mixture. Morphological traits were measured at fully developed to the plants from the transplanted stems. They were measured from 4th to 7th at every 1-week interval. Chlorophyll contents of the sweetpotato plants were measured using portable chlorophyll content meter (CL01, Hansatech Instruments) (England) and 10 points of the same leaf were measured for 4 weeks. Changes of petiole length (cm), plant height (cm), number of leaves, number of branches and stem diameter (mm) in wild type and S598 sweetpotato plants

were measured with the same leaf of each plant for 3 weeks (5th to 7th week after transplanting)

## RESULTS AND DISCUSSION

### Production of S598A transgenic plants

S598A gene was inserted into the sweetpotato genome by *Agrobacterium* mediated transformation. As the vector used this study contained NPTII gene as a selectable marker, calli were selected on medium containing G418 which is analogue of kanamycin and used for the selecting plants which exhibit intrinsic kanamycin-resistant plants (Dekeyser *et al.*, 1989; Nehra *et al.*, 1994; Cheng *et al.*, 1997; Okada *et al.*, 2001; Wakita *et al.* 2001; Howe *et al.*, 2006; Shin *et al.*, 2007). G418-resistant green sectors were transferred to regeneration medium and plants were transferred to magenta box containing soil mixture. For the selection of S598A-NPTII plants, 500 ppm of paromomycin solution was sprayed to the plants. As described by Shin *et al.* (2007), one week after spraying paromomycin, resistant plants were survived and maintained their morphology, while susceptible plants was completely bleached or necrotized and finally died (Fig. 2).

### Determination of S598A sweetpotato by PCR and Southern analysis.

Genomic DNA isolated from the wild type and S598A sweetpotato plants were analyzed using PCR. The presence of a 1-kb band in S598 plant was shown (Fig. 3A), indicating S598A gene was transferred to the sweetpotato genome, but the band was not appeared in wild type. By Southern blot analysis, we confirmed that a single copy of the gene in S598A plant stably was integrated into the sweetpotato genome (Fig. 3B). It is known that for using *Agrobacterium*-mediated transformation, low transgene copy is integrated into the plant genome compared biolstics (Tingay *et al.*, 2002; Yi *et al.*, 2007).

### Morphological traits of S598A plants

Chlorophyll content. We measured chlorophyll content leaf tissue on the 4th or 5th leaf at the similar stage of both wild type and S598A sweetpotato plants since 4 weeks after transplanting for 4 weeks. Chlorophyll content was similar at 5-week after transplanting in both wild type and S598A plant. After 6 weeks, chlorophyll content of leaf tissue in wild type plant decreased while chlorophyll content of leaf tissues in the S598A sweetpotato plants continuously increased (Fig. 4A). At 7-week after transplants, S598A plants had over two-fold higher chlorophyll content as well as darker pigmentation than the wild type plants. We found that S598A sweetpotato plant had higher chlorophyll content, was healthier and delayed leaf senescence.

Petiole length and internode length. We compared petiole length between wild type and S598A sweetpotato plant (Fig. 4B). Internode of S598A sweetpotato plant was very short and many branches were continuously generated from the internodes compared to wild type, Petiole length was measured in the marked leaf. Petiole length of wild type is continuously extended until 7 weeks after transplanting, but the petiole growth of S598A plants at this stage was stopped (Fig. 4B & Fig. 5). Thus S598A transgenic plants had shorter petiole length.

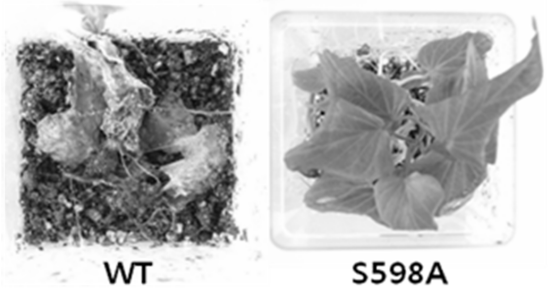



**Fig. 2.** NPTII-resistant plant selection by paromomycin spray. The plants expressing NPTII was determined by 500 ppm of paromomycin spray. WT, wild type sweetpotato; S598A, Plant expressing S598A-NPTII.

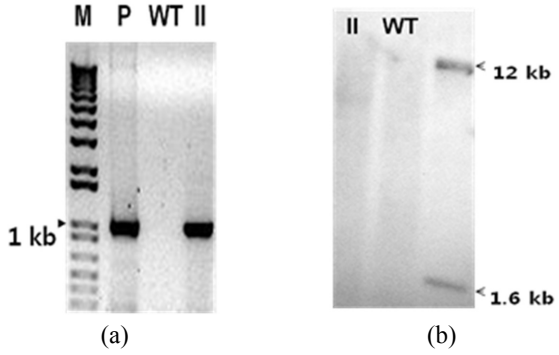
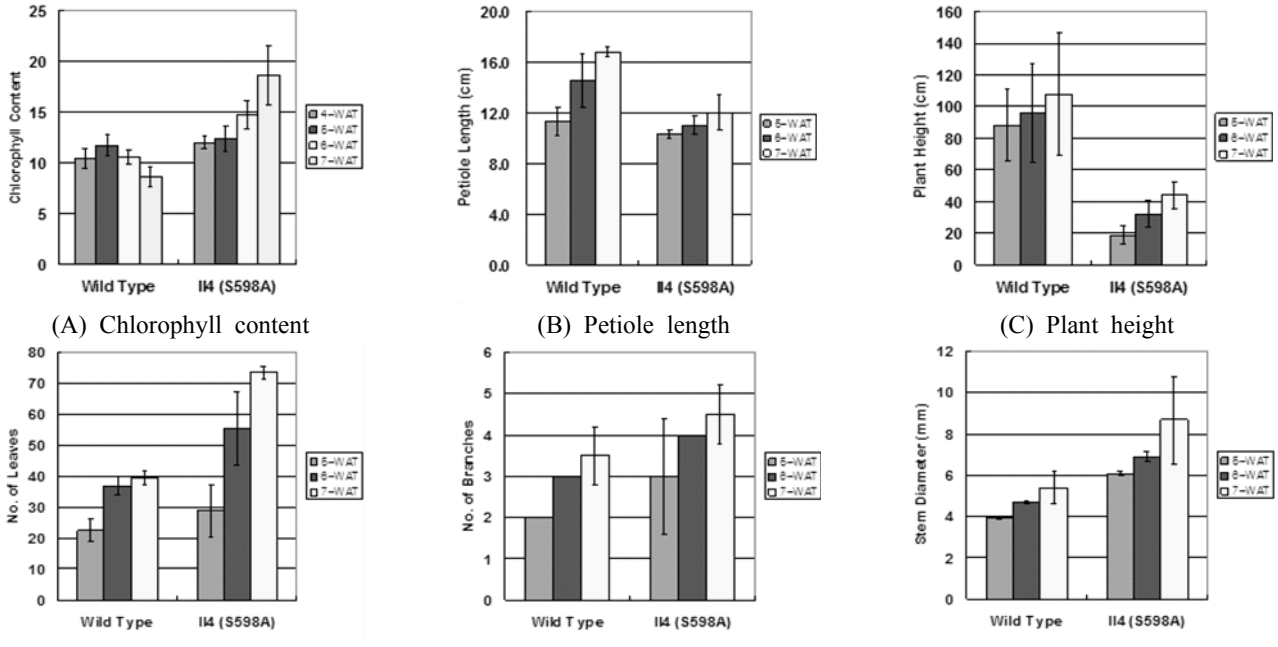



**Fig. 3.** PCR (A) and Southern blot analysis (B). M, DNA size marker; P, positive control (pCAMBIA2301-S598A); WT, wild type sweetpotato plant; II, S598A sweetpotato plant.




(A) Chlorophyll content (B) Petiole length (C) Plant height

(D) No of leaves (E) No. of branches (F) Stem diameter

**Fig. 4.** Morphological traits of wild type and S598A sweetpotato plants.

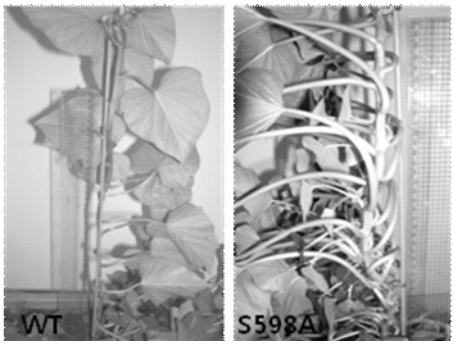



**Fig. 5.** Internode length and petiole number of wild type (WT) and S598A sweetpotato (S598A) plants (ruler : 50 cm).


**Fig. 6.** Phenotype in wild type (WT) and S598A sweetpotato (S598A) plants.

Plant height. Plant height was compared between wild type and S598A plant 7 weeks after transplanting. Ay seven weeks after transplanting, plant height of wild type plants was greater than 100 cm, but plant height of S598A transgenic plants was about 50 cm (Fig. 4C). Therefore, it is concluded that S598A plants had semi-dwarf plant height.

Number of leaves. We have measured leaf numbers from 5 to 7 weeks after transplanting. In wild type, increase of leaf numbers was almost stopped at 7 weeks after transplanting. After 7 weeks, leaves in S598A plants were continuously produced. After 7 weeks, S598A transgenic plants had 2-fold numbers of leaves than wild type (Fig. 4D).

Number of branches and stem diameter. Number of branches in S598A plants was also higher than wild type. At 7 weeks after transplanting, S598A plant had 4.5 numbers of branch (1.2-fold more branches) while wild type with 3.5 branches (Fig. 4E). S598A sweetpotato had thicker stem diameter than wild type. At 7th week, S598A plant had 8.5mm stem diameter (1.6-fold thickness) while wild type with 5.4mm in diameter (Fig, 4F).

Phenotypic characteristics of S598A plants compared with those of wild type. S598A transgenic sweetpotato had shorter plant height, darker green leave, and more numbers of leaves (Fig. 5, Fig. 6). Our study is shown to be similar result in

S598A *Arabidopsis* reported by Kim *et al.* (2004) This S598A sweetpotato plant in under field test.

In conclusion, phenotypic characteristics of S598A plants were compared with those of the wild type plant. In this study, expression of functional S598A in the sweetpotato plants resulted in pleiotropic effects related to semi-dwarfism, a higher number of leaves but smaller, and dark green leaf color with increased pigmentation. Thiele *et al.* (1999) reported that it is due to the increased numbers of chloroplasts in elongated palisade cells, photosynthesis per leaf area and in each individual plant increased. As the result of Kim *et al.* (2004) reported in *Arabidopsis*, S598A sweetpotato plant was less sensitive to photo-inactivation under prolonged light stress in photosynthesis, as a result that the senescence in S598A sweetpotato plants was delayed, and deceleration of chlorophyll degradation extended the lifetime of photosynthetically active plants. Therefore, the higher photosynthetic performance, that is, plants use sun light more efficiently, the longer lifespan and semi-dwarfism of the S598A sweetpotato plants offer a great production of biomass for bioenergy as well as starch for industrial usage.

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