Interaction of Scion and Stock on Leaf Senescence of Soybean Plants Grafted at Mid-Stem during Ripening

Taiichiro Ookawa, Noriko Tomita and Tadashi Hirasawa

(Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan)

Abstract: Leaf senescence is slower in the soybean cultivar Tachinagaha (T) than in the cultivar Enrei (E). Reciprocal grafting of the two cultivars at the basal node showed that this difference was related to roots properties. However, roots had no effect on leaf senescence at a late stage of ripening. To investigate whether the properties of the above-ground parts of plants affect leaf senescence, we grafted the two cultivars at the internode between the 8th and 9th nodes of the stem. Regarding the effect of the scion on the stock, the chlorophyll content of leaves on the E stock was maintained at a higher level when the scion was T than it was E, and the chlorophyll content of leaves on the T stock decreased faster when the scion was E than when it was T. Regarding the effect of the stock on the scion, the chlorophyll content of leaves on the T scion decreased faster when the stock was E than when it was T, although the effect of the stock on leaf senescence of the scion were weaker compared with that of the scion on the stock. Differences in photosynthetic rate were similar to those in chlorophyll content. Thus, it was clear that leaf senescence was affected by the properties of both the higher and lower above-ground parts of plants, in addition to roots.

Key words: Graft, Photosynthetic rate, Scion, Senescence, Soybean, Stock.

Leaves of the soybean cultivar Tachinagaha (T) senesce more slowly after flowering and maintain a higher rate of photosynthesis throughout the ripening period than leaves of the cultivar Enrei (E), leading to increased production of dry matter and higher yields in T plants (Ookawa et al., 1999b). Leaf senescence is affected not only by the characteristics of a shoot but also by the physiological activities of roots (Garrison et al., 1984). In previous studies (Ookawa et al., 1999a, 2001), in order to clarify which factors of roots and the above-ground parts of the plants affect the different patterns of leaf senescence in the two cultivars, we performed self-grafting and reciprocal grafting of T and E at the cotyledonary node. Root length per plant was longer in the T stock than in the E stock irrespective of the cultivars of scion throughout the ripening period. At an early stage of ripening, the exudation rates per plant and per unit leaf area, which represents the physiological activity of the root, were higher in the plants with the T stock than with the E stock, irrespective of the cultivar of scion. The differences between graft combinations were small at a late ripening stage. Thus, physiological activity was affected by the cultivar of the scion irrespective of the cultivar of the stock. As a result, leaf senescence was slower in the plants on the T stock than on the E stock at an early stage of ripening.

Leaf senescence is affected not only by the properties of the underground parts of plants, but also by the properties of the above-ground parts (Nooden and Obermeyer, 1981; Guiamet and Giannibelli, 1996). Previous studies with grafted plants (Ookawa et al., 1999a, 2001) showed that leaf senescence was largely affected by the properties of the above-ground parts of plants at the late stage of ripening. Leaf senescence is not only affected by the genes that act to maintain the chlorophyll level in leaves (Guiamet and Giannibelli, 1996), but also by plant hormones transferred from the other organs of above-ground parts to leaves and the nitrogen and other substances transferred from leaves to sink organs (Sinclair and de Wit, 1975; Nooden and Obermeyer, 1981; Craft-Brandner and Egli 1987). If the movement of these substances affects leaf senescence, it might be assumed that leaf senescence of the stock or scion is affected by the cultivar of the graft partners.

In this study, to investigate the possibility that the transferable substances are related to leaf senescence, we examined the effects of the stock and scion on the decrease of chlorophyll contents and photosynthetic rates of the leaves on the scion and stock, respectively, by reciprocal grafting of E and T plants at mid-stem internodes.

Materials and Methods

Seeds of soybean cultivars Enrei (E) and Tachinagaha

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(T) were planted with 3 seeds per pot in 300 pots (diameter 5 cm) filled with commercial soil (Shinano soil) on 18 June, 1998. Plants were grown in a greenhouse, and thinned out to one plant per pot on 2 July. Wedge-grafting was performed at the center of the internode between the 8th and 9th node during the emergence of the 10th leaf on 22 July. The self-grafted plants of two cultivars, which served as controls for the grafting operation, were referred to as T/T and E/E, respectively. The reciprocally grafted plants of the two cultivars were referred to as T/E and E/T. About 100 plants of each graft combination were grafted. The plants after grafting were grown under 50% shade in a mist room (at least 90% humidity) to promote graft union for one week. Vigorously growing grafted plants were chosen from each graft combination and transferred to 1/2000 a pots on 31 July. Ungrafted E and T plants were also grown under the same conditions. Compound fertilizer (14-14-14) was added to each pot (2 g pot\(^{-1}\)) at the time of transplanting.

Grafting at the internode between 8th and 9th nodes of the stem was successful in 40%, 32%, 31% and 70% of the E/E, T/E and E/T plants, respectively. Although the success rate of grafting was lower in the combination of different cultivars than in that of the same cultivar, the leaves of the scion in successfully grafted plants expanded well and plant growth was not disturbed by grafting.

Efforts were made to prevent damage by insects and disease. The beginning of flowering was on 26 July in both grafted and ungrafted plants. For measurements of various items, grafted plants that possessed 5 nodes of the scion were used.

The chlorophyll content of leaves was estimated at the central region of terminal leaflets of compound leaves at each node on the main stem with an in situ chlorophyll meter (SPAD-502, Minolta Inc., Tokyo). Readings of the chlorophyll meter were correlated closely to the chlorophyll contents determined by the method of Wintermans and Mots (1965).

The photosynthetic rate was measured at the terminal leaflets of compound leaves on the main stem with a portable photosynthetic and transpiration system (LI-6200, LI-COR) under outdoor conditions where the plant grew. It was measured between 0800 and 1200 h at the ripening stage. The intensity of light at the leaf surface was over 1,200 \(\mu\text{mol m}^{-2} \text{s}^{-1}\), and the concentration of CO2 in the chamber at the start of the measurement was 350 \(\mu\text{L L}^{-1}\).

**Results**

1. **Comparison of growth and leaf senescence between ungrafted Enrei and Tachinagaha cultivars**

The mean final length of the main stem of ungrafted plants at 56 days after the beginning of flowering (DABF) was 62 cm in the E plants and 52 cm in the T plants. The mean final number of nodes on the main stem was 13 in both cultivars.

At 17 DABF, there were no differences between the cultivars in the chlorophyll content of leaves at any node except for the leaves at the 2nd node (Fig. 1). At 34 DABF, there were no differences in the chlorophyll content of leaves at the 8th to 13th node, but the chlorophyll content of leaves at lower nodes (2nd, 5th and 6th nodes) was lower in the E plants than in the T plants. At 49 DABF, the late stage of ripening, the chlorophyll content of leaves at the 3rd to the 8th nodes was clearly lower in the E plants than in the T plants except for the 5th node. At 60 DABF, the leaves at the 2nd and 3rd nodes of the T plants had been

![Fig. 1. Changes in the chlorophyll contents of leaves in ungrafted plants after the beginning of flowering.](image-url)

○ : Enrei, ● : Tachinagaha

The 1st node refers to the cotyledonary node. The 13th node is the uppermost node with the 11th foliage leaf. Values are means and standard deviations from six plants. Vertical bars indicate the standard deviations. The relative value of chlorophyll content indicates the reading from the chlorophyll meter. Zero values were given for the shed leaves.
shed, but the chlorophyll content of leaves at the upper nodes was well maintained. By contrast, in the E plants, the leaves at the 2nd to 10th nodes had already been shed, and the chlorophyll content of leaves at the 11th and 12th nodes was lower in the E plants than in the T plants. We confirmed that the leaves of the E plants senesced more rapidly than those of the T plants (Ookawa et al., 1999a, 1999b).

2. Comparison between graft combinations

(1) Plant growth

The mean length of the main stem in the grafted plants of the four combinations ranged from 56 to 60 cm. The mean number of branches and nodes on the main stem in the grafted plants ranged from 8.0 to 9.3 and from 13.2 to 13.3, respectively, with no significant differences in these growth parameters between the graft combinations (Table 1).

There were also no differences between the graft combinations in the leaf area of the scion and stock at 16 DABF. The leaf area of the scion was larger in T/T and E/T than in E/E and T/E, but there were no differences between the graft combinations in the leaf area of the whole plants (Table 2). No differences were found between the graft combinations in either grain weight of the scion or stock on the main stem (Table 3). Thus, there were no differences in growth between the graft combinations.

(2) Degree of leaf senescence

1) Chlorophyll content

a. The effect of scion on the leaf senescence of stock

The effect of difference in the cultivar of the scion on leaf senescence of the stock was investigated (Fig. 2). At 16 DABF, the chlorophyll content of the leaves at the lower nodes of the stock was lower in the E stock than in the T stock, but the effects of the scion on the leaf senescence of the stock were not apparent. At 26 and 34 DABF, the chlorophyll content of the leaves of the E stock was higher in T/E than in E/E. At 53 and 60 DABF, almost all leaves had already been shed from the E stock.

Until 53 DABF, the effect of the scion on leaf senescence of the T stock was slight. At 60 DABF, the chlorophyll content in leaves of the stock was remarkably lower in plants with the E scion (E/T) compared to those with the T scion (T/T).

b. The effect of stock on leaf senescence of the scion

The effect of difference in the cultivar of the stock on leaf senescence of the scion was investigated (Fig. 2).

At 16, 26 and 34 DABF, no differences in the chlorophyll content in leaves of the scion were observed between the graft combinations (Fig. 2). At 53 DABF, late stage of ripening, the decrease in chlorophyll content of the leaves was larger in plants with the E scion than with the T scion, and differences in the chlorophyll content of leaves appeared between the graft combinations. In plants with the T scion, the extent of decrease in the chlorophyll content at the lower nodes of the scion was larger in plants with the E stock (T/E) than in plants with the T stock (T/T). At 60 DABF, the differences in chlorophyll content of leaves between the scions of T/E and T/T became clear. However, the difference between the E scions of E/T and E/E was not clear at 53 and 60 DABF, except the difference in the chlorophyll content of leaves at the 10th node between E/E and E/T at 53 DABF.

2) Photosynthetic rate

The photosynthetic rate of leaves at individual nodes was compared between the graft combinations at 53 and 61 DABF (Fig. 3). Large differences in the photosynthetic rate of leaves were found between the graft combinations as for the chlorophyll content. The
decline of the photosynthetic rate of leaves in both the T scion and T stock was larger in the plants grafted with Enrei than in the plants grafted with Tachinagaha, but the difference in the photosynthetic rate was not observed in the E scion.

Discussion

In this study, to clarify the factors causing different patterns of leaf senescence in a late-senescing cultivar, Tachinagaha (T), and an early senescing cultivar, Enrei (E), we grafted T and E at the mid-stem (internode between the 8th and 9th node), and examined the effects of both the scion and stock on leaf senescence of the graft partners. The grafted plants grew successfully, and there were almost no differences between the graft combinations in the final length of the main stem, the final number of branches, the final number of pods, the leaf area of both the scion and stock and the grain weight of the scion and stock (Tables 1 and 2). Therefore, the differences in these growth parameters between the graft combinations might not affect leaf senescence directly.

In the ungrafted plants, leaf senescence was earlier in the E plants than in the T plants; and in the self-grafted plants, leaf senescence of both the scion and stock was earlier in E/E than in T/T (Figs.1 and 2). In our previous studies (Ookawa et al., 1999a, 2001), the same differences in leaf senescence were observed between control and grafted plants by a wedge-grafting at the cotyledonary node as those found in this study by wedge-grafting at a higher internode of the stem. Thus, we consider that the artificial grafting does not affect the leaf senescence directly.

It is clear that the properties of the scion and stock affected leaf senescence of the stock and the scion, respectively (Fig. 2). Regarding the effect of the scion on leaf senescence of the stock, leaf senescence of the E stock was slower in T/E than in E/E, indicating that leaf senescence of the stock was affected by the cultivar of the scion. This indicated that leaf senescence of the stock was also affected by the scion at an early stage of ripening, although it was strongly affected by the underground parts of the plant as observed in the plants grafted at the cotyledonary node (Ookawa et al., 2001). Furthermore, leaf senescence at the lower node of the T stock was earlier in E/T than in T/T.

Besides, there were large differences in the leaf senescence between E scion and T scion at a late stage of ripening. Leaf senescence of the T scion was markedly earlier in T/E than in T/T, although leaf senescence of the E scion was less affected by the stock. The reason why the leaf senescence of the E scion was less affected by the cultivar of the stock has not been clarified, but these results suggest that substances that suppress or accelerate leaf senescence move from a stock to a scion or vice versa. In our previous study (Ookawa et al., 1997), in order to distinguish the effects of the above-ground parts and the...
underground parts of the stock on the leaf senescence, we performed self-grafting and reciprocal grafting of T and E plants at the two positions of the stem, cotyledonary node and higher node of stem. The leaf senescence of the T scion was accelerated by the above-ground parts of the E plant, irrespective of the cultivar of the underground parts. This means that the leaf senescence of the scion is affected by the substances transferred from the above-ground parts of the plants. There is a possibility that plant hormone-like substances that delay leaf senescence are transported across the graft union. In grafting experiments with peas, the presence of a graft-transmissible factor that delayed leaf senescence has been reported (Proebsting 1977).

Cytokinin (Garrison et al., 1984), gibberellin (Nooden 1986, Sklensky and Davis 1993) and auxin (Nooden et al., 1979, Nooden and Nooden 1985) have been known as senescence-retarding plant hormones. Leaf senescence is delayed by cytokinins, which are synthesized in roots and transported to the above-ground parts of the plant via the xylem. Leaves can also synthesize cytokinins (Singh et al. 1992). Auxin is synthesized in young leaves, apical buds of stems, and immature seeds (Sweetser and Swartzfager 1978). Auxin is transported to the other parts of plants, and acts to suppress leaf senescence and abscission (Nooden et al. 1979). Such senescence-retarding plant hormones might affect the leaf senescence in the plants grafted with Tachimagaha (Fig.2).

Leaf senescence of both the T scion and T stock was accelerated by grafting with Enrei (Fig. 2), indicating that leaf senescence is strongly affected by the substances from the early-senescing cultivar, Enrei. Abscisic acid (ABA) and ethylene are plant hormones that accelerate leaf senescence. ABA accumulates in leaves of field-grown soybean plants during leaf senescence (Samat and Sinclair 1980). Leaf senescence is delayed in ethylene-deficient transgenic tomato plants to which a 1-aminocyclopropane-1-carboxylate synthase gene has been introduced (Jaglo-Ottosen et al. 1992).

The photosynthetic rates of leaves among grafted plants at 53 and 61 days after the beginning of flowering are shown in Fig. 3. The photosynthetic rates were measured using a portable photosynthesis system (LI-6400, LI-COR, Inc.). The values are means and standard deviations from three plants. Vertical bars indicate the standard deviations.
acid (ACC) oxidase antisense gene was introduced. On the other hand, ethylene generated in the above-ground parts of the wild-type plants promotes leaf senescence (John et al. 1995).

In addition to plant hormones, the amount of nitrogen in the plant might affect leaf senescence. Nitrogen is the primary component of proteins, which include ribulose-1,5-bisphosphate carboxylase/oxygenase and other photosynthesis-associated enzymes. In soybean plants, it was suggested that the translocation of nitrogen from leaves to pods might be one of the factors of leaf senescence (Sinclair and de Wit, 1975). In this study, there seems to be no difference in the sink size of the scion and stock between the graft combinations because the number of pods was almost the same. However, a large amount of nitrogen is accumulated in the leaves and stems compared with the pods in Tachinagaha at the ripening stage (Kakubari et al., 2003). There is a possibility that these properties of cultivars contributed to the differences in the amount of nitrogen in the scion and stock between different graft combinations. Further research on the relationship between leaf senescence and the allocation of nitrogen in plants, focusing on varietal differences, is necessary.

Further studies are under way to identify the transferable substances that cause the varietal difference in leaf senescence, including synthesis of physiologically active substances that modulate leaf senescence and movement of these substances in the above-ground parts of soybeans.

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