Anatomy and Physiology of Graft Incompatibility in Sturt’s Desert Pea (Swainsona formosa), an Australian Native Plant

Masayo Kawaguchi and Acram Taji
School of Rural Science and Agriculture, University of New England
Armidale, NSW, 2351
Australia

Keywords: vascular tissue, inorganic mineral nutrient, breaking weight, peroxidase, CO₂

Abstract

The mechanisms and symptoms of graft incompatibility were investigated using Sturt’s Desert Pea (SDP, Swainsona formosa) grafted onto SDP, Parrot’s Bill (PB, Clianthus puniceus), Senna artemisioides and Acacia terminalis. From the results of previous experiments, SDP/PB and SDP/SDP were regarded as graft compatible combinations, and SDP/Senna and SDP/Acacia were regarded as graft incompatible combinations. Four weeks after grafting, continuing vascular tissues from rootstocks to scions were observed at the graft unions of compatible combinations. With incompatible combinations, parenchyma cells (calli) were observed between vascular bundles of rootstocks and those of scions, and no vascular connections were observed at the graft unions. At 4 weeks after grafting, increased fresh weight, dry weight, weight ratio and concentrations of inorganic mineral nutrients were obtained in scions of compatible combinations, compared to those of incompatible combinations. The breaking weight of the graft union of compatible combinations was bigger than that of incompatible combinations. Increased peroxidase activities were obtained in SDP scions grafted onto incompatible rootstocks. It was discovered that higher CO₂ concentrations occurred around grafted plants of compatible combinations than those of incompatible combinations.

INTRODUCTION

Many researchers have reported that for grafting success, proliferation of callus both from the rootstock and the scion, differentiation of new vascular cambium from callus cells, differentiation of secondary xylem and phloem, and transport of water and nutrients through the secondary xylem and phloem were essential (Stoddard and McCully, 1979; Moore and Walker, 1981; Gebhardt and Goldbach, 1988). Incompatible graft combinations are thought to have problems in some stages of graft union formation, though the mechanism of graft incompatibility is still not clear. Some researchers have reported that peroxidase activity was involved in the graft union formation (Buchloh, 1960; Santamour, 1988).

In previous experiments, around 50% of Sturt’s Desert Pea (SDP, Swainsona formosa) grafted onto SDP and Parrot’s Bill (PB, Clianthus puniceus) grew well, while SDP grafted onto Senna artemisioides and Acacia terminalis did not grow at all (Kawaguchi and Taji, 2002). Therefore, SDP/PB and SDP/SDP were regarded as graft compatible combinations, and SDP/Senna and SDP/Acacia were regarded as graft incompatible combinations. To determine the reason for graft incompatibility in SDP grafting, differences between graft compatible combinations and graft incompatible combinations were investigated, from both physiological and anatomical perspectives, using these graft combinations.

In this experiment, water stress, concentrations of inorganic mineral nutrients and peroxidase activity in scions were investigated, and vascular tissue connections between the scion and the rootstock were observed. Lindsay et al. (1974) used breaking weight apparatus at the graft union to evaluate the grafting success. Respiration is arguably the most important physiological activity for plants, and measuring carbon dioxide concentration is an effective method by which to investigate this activity. Therefore,
breaking weight of the graft union was measured using the method of Lindsay et al. (1974), and CO₂ concentrations around grafted plants were also analysed in this experiment.

MATERIALS AND METHODS

Four graft combinations; SDP/SDP, SDP/PB, SDP/Senna artemisioides and SDP/Acacia terminalis, and non-grafted SDP (control) were used. Each seed was sown directly onto 10 ml of MS basal medium (Murashige and Skoog, 1962) in a 30 ml culture vessel. Seedlings were grown under light conditions with 20 µmol m⁻² s⁻¹ light intensity for 16 hours a day. Temperature was kept at 25°C. Four weeks later, seedlings were grafted under in vitro conditions. The stem of the rootstock was cut horizontally, with a scalpel, below the cotyledon, and was then notched. The stem of the scion was cut above the cotyledon and the surface was cut into a wedge-shape. All leaves except a few were removed from the scions, and the scions were inserted into the rootstocks. Each grafted plant was transferred into 30 ml of MS basal medium in a 120 ml culture vessel. The lid of culture vessel was further sealed by a strip of Parafilm® wrapped around the lid. Grafted plants were placed in a plant growth cabinet set at 20°C, under a 16 hour photoperiod with light intensity of 20 µmol m⁻² s⁻¹. Four weeks after grafting, well grown SDP/SDP and SDP/PB and surviving SDP/Senna and SDP/Acacia were harvested for analysis (see following). Control seedlings were grown using the same methods as grafted plants, except that they were not grafted. For the control, the part of the stem where the cotyledons were attached was regarded as the graft union. Parts above the cotyledons were regarded as the scions, and the part of the plant below the cotyledons was regarded as rootstocks.

Distribution of Vascular Tissues at the Graft Union

Most of the methods used here were from Prakash (2000). Four weeks after grafting, grafted parts were sampled, kept in formalin acetic alcohol for 48 hours, and prepared by dehydration, infiltration, mounting and sectioning (longitudinally with 20 µm thickness), and then placed on glass slides. Sections were stained with 1% aqueous safranin for 6 hours and 0.5% fast-green in 95% ethanol for 15 seconds. Slides were observed using a light microscope and photographed by an Olympus camera.

Water Stress and Concentrations of Inorganic Mineral Nutrients

Four weeks after grafting, grafted plants were collected and fresh weight was measured. After the plants were kept in an oven at 80 °C for 2 days, dry weight of the plants was measured, and the fresh/dry weight ratio was calculated. Then, concentrations of inorganic mineral nutrients were analysed.

Dried samples were ground and weights of 0.2 g for scions and 0.15 g for rootstocks were measured out. Digestion of the tissue was undertaken using a method adapted from Anderson and Henderson (1986). Concentrations of inorganic mineral nutrients (K, Mg, Ca, P, S, Na, B, Fe, Cu, Mn and Zn) were analysed using ICP (Inductively Coupled Plasmas, Varian, Vista-MPX™ CCD simultaneous ICP-OES).

Measuring the Breaking Weight of the Graft Union

The strength of the graft union was measured using the method of Lindsay et al. (1974). The breaking weight of the graft unions was determined by applying a weight (sand) that increases at a constant rate through the long axis of the grafted internodes.

Total Peroxidase Determination

Method of peroxidase extraction was adapted from Gillikin and Graham (1991), Hammerschmidt et al. (1982) and Rathmell and Sequeira (1974). Fresh weight, 0.1-0.5 g, of plant material was used for each sample. Peroxidase activity was assayed using the methods of Gillikin and Graham (1991). Peroxidase activity was measured spectrophotometrically (Milton Roy Spectronic 20D spectrophotometer) at 470 nm at
Analysis of CO₂ Concentration

For this analysis, vessels with modified lids were used for incubation of grafted plants. Septa (diameter 85 mm) were mounted in the middle of the lids. The concentration of CO₂ around the grafted plants was analysed at 1, 2, 3 and 4 weeks after grafting. The air in the vessel (120 ml) which contained the grafted plant was mixed by gently rotating the culture vessel and by drawing the air several times into an empty syringe and expelling the air. A 500 µl sample was taken by passing the needle of a gas tight syringe through the septa. CO₂ concentration was analysed using a HP6890 gas chromatography fitted with a HP5973 mass selective detector. Column type and carrier gas were HP-5MS (5 % phenyl methyl siloxane) and helium (0.8 ml/min), respectively.

RESULTS

Results of anatomical work (Fig. 1) showed that vascular tissues of the SDP scions were connected to the vascular tissues of the SDP and PB rootstocks at 4 weeks after grafting. The vascular tissues of Senna and Acacia rootstocks were not connected to those of the SDP scions at 4 weeks after grafting. Parenchyma cells (calli) were observed between the vascular tissues of the Senna and Acacia rootstocks and those of the SDP scions.

Fresh weight (FW), dry weight (DW), weight ratio (FW/DW) and concentrations of inorganic mineral nutrients (K, Mg, Ca, P, S, Na, B, Fe, Cu, Mn and Zn) of SDP scions grafted onto SDP, PB, Senna and Acacia rootstocks and non-grafted SDP at 4 weeks after grafting, are shown in Table 1. At the time of grafting, the size of the scion was uniform for all the graft combinations. Increased fresh weight, dry weight and weight ratio were obtained in SDP grafted onto PB and SDP and non-grafted SDP at 4 weeks after grafting. Conversely, fresh weight, dry weight and weight ratio of scions in SDP/Senna and SDP/Acacia showed small values at 4 weeks after grafting. Trends showed that higher concentrations of inorganic mineral nutrients were found in the scions of SDP/PB and SDP/SDP and non-grafted SDP, compared to the scions of SDP/Senna and SDP/Acacia at 4 weeks after grafting, although there were some exceptions.

The breaking weights of grafted plants and non-grafted plants are shown in Table 2. The breaking weights of the graft unions of SDP/Senna and SDP/Acacia were similar, and they decreased greatly compared to those of non-grafted SDP. The breaking weights of the graft unions of SDP/SDP and SDP/PB could not be measured exactly because non-grafted parts of stem were broken before grafted parts were broken for all SDP/PB plants and some of the SDP/SDP plants.

Fig. 2 shows that peroxidase activities in SDP scions grafted onto Senna and Acacia rootstocks were significantly higher than those in SDP scions grafted onto SDP and PB and those in the non-grafted plants. There were no differences in peroxidase activity between non-grafted plants above the cotyledons and SDP scions grafted onto SDP and PB rootstocks.

Fig. 3 shows CO₂ concentration around grafted SDP. The CO₂ concentration around SDP grafted onto both SDP and PB was higher than that around SDP grafted onto both Senna and Acacia for the entire duration of the experiment. The CO₂ concentrations around SDP/Senna and SDP/Acacia were similar.

DISCUSSION

Continuous vascular tissues from rootstocks to scions were observed at the graft unions of graft compatible combinations (SDP/SDP and SDP/PB), and no vascular connections were observed at the graft unions of graft incompatible combinations (SDP/Senna and SDP/Acacia). Parenchyma cells (calli) were observed between the vascular tissues of the incompatible rootstocks and those of the SDP scions. Therefore, SDP/Senna and SDP/Acacia seemed to have problems with differentiation of cambium and vascular tissue from calli at the graft union, and this is suspected to be the reason for
graft failure.

Trends show that increased fresh weight, dry weight, weight ratio and concentrations of inorganic mineral nutrients were obtained in scions of graft compatible combinations, compared to graft incompatible combinations. Continuous vascular tissues between the rootstock and the scion were thought to lead to increased fresh weight, dry weight, weight ratio and concentrations of inorganic mineral nutrients in graft compatible combinations. Conversely, in graft incompatible combinations, discontinued vascular tissues between the rootstock and the scion may lead to water stress and lower concentrations of inorganic mineral nutrients in scions, preventing any size increase in scions after grafting. However, the species of rootstock also affects the concentrations of inorganic mineral nutrients in scion. For example, higher concentration of P was obtained in SDP scion when grafted onto Acacia, because generally Acacia species absorb high concentrations of P from the substrate. Increased concentrations of P in SDP scion is due to transfer of P ions through the callus cells as a result of osmosis.

Differentiation of the vascular tissue, especially xylem, at the graft union contributes to mechanical support of the graft union. Moore (1982) reported that three phases of cohesion between the scion and the rootstock were observed during the graft union formation of compatible autografts in Kalanchoe blossfeldiana, and that the second phase of cohesion was correlated with an integration of callus cells at the graft interface and differentiation of vascular tissue across the graft interface.

The breaking weights of the graft unions in graft incompatible combinations decreased greatly, compared to those in non-grafted plants at 4 weeks after grafting. The lack of differentiation of vascular tissue at the graft union was concluded to contribute to the lower breaking weight in incompatible combinations. As the breaking weights of the graft unions in graft compatible combinations were much higher, non-grafted parts were broken before grafted parts in all SDP/PB plants and some of the SDP/SDP plants at 4 weeks after grafting. This indicates that mechanical restoration of the graft union was complete within 4 weeks after grafting for these graft compatible combinations.

Lignin is a component of xylem, and lignin may greatly contribute to the cohesion between the scion and the rootstock, leading to a strong graft union. Peroxidases catalyse the polymerisation of cinnamic alcohols into lignin (Hammerschmidt et al., 1982). Therefore, increased peroxidase activities were expected in graft compatible combinations (SDP/SDP and SDP/PB). However, results obtained in SDP scions in this experiment contradicted this hypothesis. Peroxidase activity in SDP scions of incompatible combinations was significantly higher than that of compatible combinations at 4 weeks after grafting.

From this result, it was assumed that the restoration of xylem was complete and higher peroxidase activities were no longer required at 4 weeks after grafting for graft compatible combinations. This assumption was also supported by the result showing that there were no differences in peroxidase activity between non-grafted plants above the cotyledons and SDP scions grafted onto compatible rootstocks. Moreover, it is hypothesised that SDP scions grafted onto incompatible rootstocks were stressed at 4 weeks after grafting, requiring increased enzymatic activity. It has been reported that increased peroxidase activity was obtained in plants under stress (Singel and Singel, 1986).

Peroxidases occur as large numbers of isozymes and participate in numerous physiological activities. Therefore, further critical investigation of the peroxidase activity particular to lignin synthesis is required, by focusing on only graft union rather than whole scion. Anodal isoperoxidase reportedly regulates lignification, which can be related to graft compatibility (Wolter and Gordon, 1975). Santamour (1988) suggested that matching of cambium isoperioxidase bands between the scion and the rootstock was necessary for grafting success.

Higher CO₂ concentrations were obtained around graft compatible combinations than around graft incompatible combinations for the entire duration of the experiment. The concentration of CO₂ is generally determined by the rates of respiration and
photosynthesis. Leaf area and rates of respiration and photosynthesis are proportional. Therefore, higher CO₂ concentrations emitted from SDP plants grafted onto both SDP and PB may be due to their increased leaf areas. However, from our previous experiment, many of the graft compatible combinations (SDP/SDP and SDP/PB) showed the size (leaf area) increase from the second week after grafting (Kawaguchi and Taji, 2002). At the end of the first week after grafting, leaf areas of grafted plants were assumed to be the same for all graft combinations. Therefore, higher CO₂ emission from SDP/SDP and SDP/PB cannot be a function of leaf area during the first week.

Considering the process of graft union formation, during the first week, the compatible graft combinations of SDP should have been going through most of the processes of graft union formation (necrotic layer formation, callus proliferation and vascular differentiation), while the incompatible graft combination of SDP should have been going through only necrotic layer formation and callus proliferation. Restoration of the graft union requires metabolic energy. This may indicate that more metabolic energy is required for graft compatible combinations to undergo these many processes, resulting in higher respiration rates during the first week post-grafting. Additionally, compatible graft combinations may have already started cell division at their apical meristems during the first week post-grafting in preparation for the later size increase, resulting in higher respiration rates.

CONCLUSIONS

The results of work presented here show the following trends: 1. Discontinuous vascular connection at the graft union is thought to decrease the water and mineral uptake from roots, preventing any size increase in scions of graft incompatible combinations. 2. Increased peroxidase activity in SDP scions of graft incompatible combinations may be caused by the stress of the lack of water and mineral nutrients. 3. The existence of the vascular tissues, especially xylem, at the graft union of compatible combinations is thought to contribute to mechanical support of the graft union. 4. An increased respiration rate was measured in graft compatible combinations, compared to graft incompatible combinations.

However, the results presented here are those of 4 or 5 replications. Further experiments with larger number of plants are required to substantiate these findings.

Literature Cited


### Tables

Table 1. Fresh and dry weight, weight ratio and concentrations of inorganic mineral nutrients of Sturt’s Desert Pea (SDP) scions grafted onto SDP, Parrot’s Bill (PB), *Senna artemisioides* and *Acacia terminalis* at 4 weeks after grafting. As a control, non-grafted SDP above the cotyledons was used. Five plants were used for measuring weight, and three to five replicates were used for analysing inorganic mineral nutrients for each scion/rootstock combination. The same letters indicate no statistical difference among the scions at a 5% level of significance, using Fisher’s LSD.

<table>
<thead>
<tr>
<th>Rootstock species</th>
<th>Weight (g) Fresh</th>
<th>Weight (g) Dry</th>
<th>Wt ratio FW/DW</th>
<th>Concentrations (%) of inorganic mineral nutrients in sample dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>K</td>
</tr>
<tr>
<td>Control</td>
<td>0.309 b</td>
<td>0.0276 a</td>
<td>11.2 b</td>
<td>5.5 a</td>
</tr>
<tr>
<td>SDP/SDP</td>
<td>0.246 b</td>
<td>0.0204 b</td>
<td>12.1 b</td>
<td>4.2 b</td>
</tr>
<tr>
<td>SDP/PB</td>
<td>0.496 a</td>
<td>0.0244 ab</td>
<td>20.3 a</td>
<td>5.5 a</td>
</tr>
<tr>
<td>SDP/Senna</td>
<td>0.069 c</td>
<td>0.0087 c</td>
<td>7.8 c</td>
<td>3.4 c</td>
</tr>
<tr>
<td>SDP/Acacia</td>
<td>0.062 c</td>
<td>0.0079 c</td>
<td>7.8 c</td>
<td>3.9 c</td>
</tr>
</tbody>
</table>

Table 2. Breaking weights (g) of graft unions were measured at 4 weeks after grafting. As a control, breaking weights of stems with cotyledons attached were measured in non-grafted SDP. Five plants were used for each scion/rootstock combination. The same letters indicate no statistical differences at a 5% level of significance, using Fisher’s LSD.

<table>
<thead>
<tr>
<th>Rootstock species</th>
<th>Control</th>
<th>SDP</th>
<th>PB</th>
<th>Senna</th>
<th>Acacia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breaking weight (g)</td>
<td>267 a</td>
<td>217 b</td>
<td>253 a</td>
<td>126 c</td>
<td>131 c</td>
</tr>
</tbody>
</table>

1 non-grafted parts were broken before grafted parts were broken for all 5 plants.

2 non-grafted parts were broken for 2 plants, and grafted parts were broken for 3 plants.
Fig. 1. Vascular bundle connections between scions and rootstocks at 4 weeks after grafting. Sturt’s Desert Pea (SDP) was grafted onto SDP, Parrot’s Bill (PB), *Senna artemisioides* and *Acacia terminalis*. At 4 weeks after grafting, graft unions were collected, and cut longitudinally with 20µm thickness, and stained with safranin and fast-green. ‘VB’, ‘SVB’ and ‘RVB’ represent vascular bundle, vascular bundle of scion and vascular bundle of rootstock, respectively. ‘P’ represents parenchyma cells (calli). (1) Graft unions of SDP/SDP (2) Graft unions of SDP/PB (3) Graft unions of SDP/*Senna* (4)(5) Graft unions of SDP/*Acacia* (x100)
Fig. 2. Peroxidase activity in Sturt's Desert Pea (SDP) scions grafted onto SDP, Parrot's Bill (PB), *Senna artemisioides* and *Acacia terminalis* at 4 weeks after grafting. Control represents non-grafted SDP. Five replicates were used for each scion/rootstock combination. Peroxidase activity was expressed as absorbance increase/min/gram fresh weight. The same letters on each bar indicate no statistical differences among the scions at a 5% level of significance, using Fisher’s LSD.

![Bar graph showing peroxidase activity](image1)

Fig. 3. CO₂ concentration of gas in the 120 ml culture vessel in which grafted Sturt's Desert Pea (SDP) was incubated for 1, 2, 3 and 4 weeks after grafting. SDP was grafted onto SDP, Parrot's Bill (PB), *Senna artemisioides* and *Acacia terminalis*. Results shown are the mean and SE for 4 grafted plants for each scion/rootstock combination.

![Graph showing CO₂ concentrations](image2)