A Grafting Method to Increase Survival of Seedlings of Interspecific Hybrids Within the Genus, Beta

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For several years there has been intermittent interest displayed in three species of *Beta;* namely *patellaris, procumbens,* and *webbiana*, often referred to collectively as the section, *patellares.* It has been shown on several occasions that these three species all exhibit resistance or immunity to curly top, leaf spot and to attack by the sugar beet nematode, *Heterodera schachtii.* A few investigators (1, 2, 4 and 5)[2] have reported their results with crossing plants of these species to *Beta vulgaris.* Results have varied, but as yet no one has been able to successfully combine the resistance from any of the members of this group with the other desirable qualities of the sugar beet, *Beta vulgaris.* The main reason for this is apparently the seedling lethal condition of the F_1 hybrids between *Beta vulgaris* and these other species. Stewart (5) reported the survival of a few seedlings of a cross between a sugar beet and *B. procumbens* and their propagation to seed, but was unable to maintain the progeny through later seed generations and the material was lost. Gaskill (2) has reported the survival of a few seedlings from crosses of swiss chard to *B. webbiana* and *B. procumbens.* He also reports that such F_1 plants exhibit resistance to the sugar beet nematode. Oldemeyer (4) has obtained a few viable plants from crosses between members of the species, *Beta vulgaris,* other than the sugar beet, and plants in the section, *patellares.*

In all the above methods of attempting to propagate the plants derived from these interspecific crosses, the survival of the F_1 plants was, at best, poor. Coe (1) studied a grafting technique to improve this survival. This consisted of grafting the small F_1 seedlings while still alive into the apex of very young sugar beet seedlings. The survival he obtained was about 7 percent.

The study being reported here is also of a grafting technique which has given a higher percentage of survival. F_1 hybrids were produced in the greenhouse by open pollinating male sterile sugar beet flowers with pollen from plants of *B. patellaris, B. webbiana,* and *B. procumbens.* Hybrid seed was readily produced in this manner. Some contamination occurred from sugar beet pollen from a nearby flowering sugar beet but the resulting seedlings could be readily identified from the hybrids. The hybrid seedlings emerged later, grew much more slowly after emergence, and all had red flecked cotyledons. None of the hybrids from these crosses grew to maturity. Some plants died in the cotyledon stage; some progressed to the two true-leaf stage; but none survived beyond the eight-leaf stage. Several nutrient solutions including rooting hormones failed to stimulate the development of roots on any of these seedlings.

It was at this point that the possibility of grafting for prolonging the lives of these hybrids was investigated. Many types of grafts were attempted and most of them failed to give satisfactory survival percentages of the

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[2] Numbers in parentheses refer to literature cited.

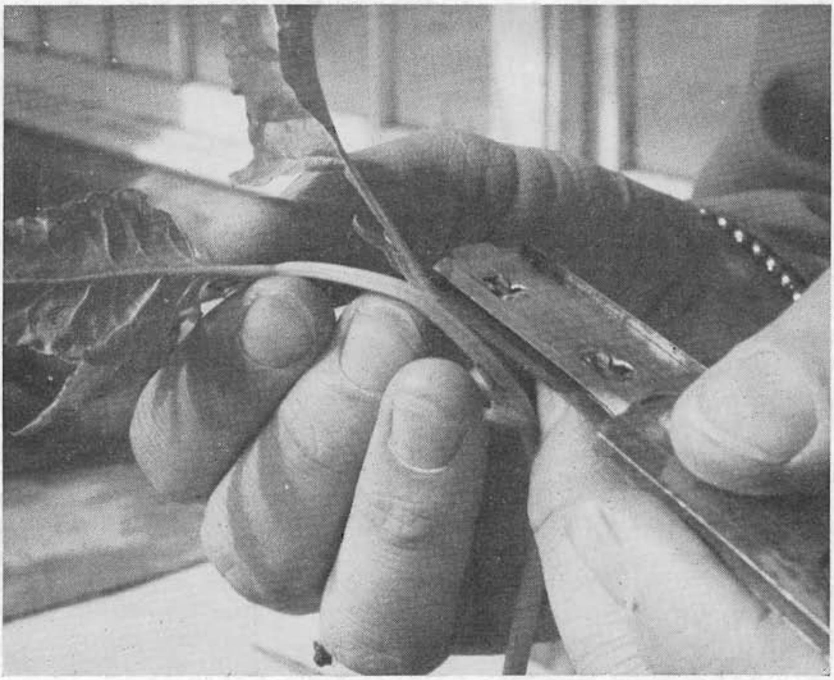

Figure 1.—A leaf and axillary bud are cut away from stem of the stalk in a vegetative area below the area of elongation, usually 3 to 4 inches below the apex.

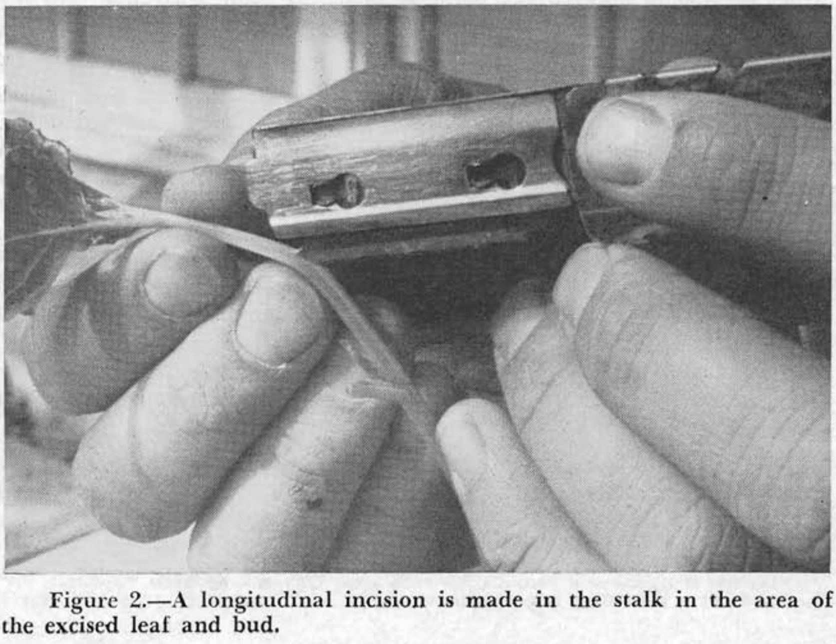

Figure 2.—A longitudinal incision is made in the stalk in the area of the excised leaf and bud.

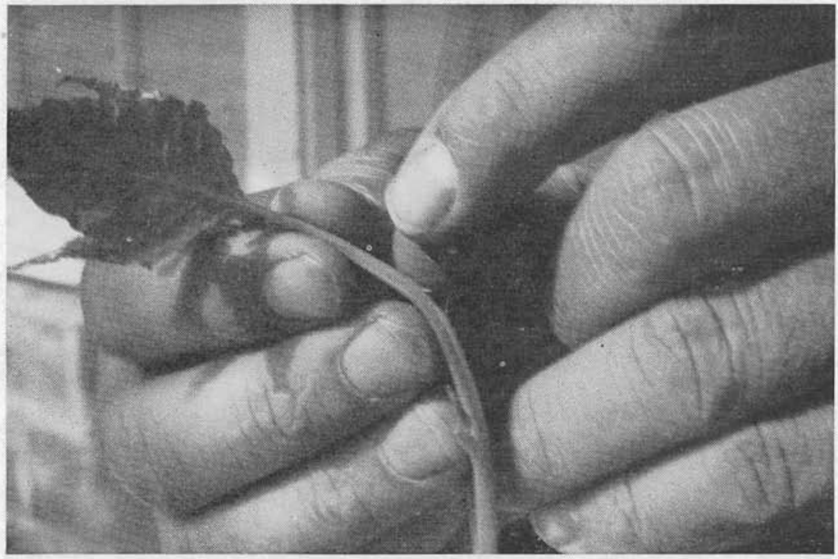

Figure 3.—The tapered scion is inserted into the incision on the stalk. Bending the stalk helps keep the incision open and prevents damage to the scion while being fitted into incision.

seedlings. One method, however, was an exception and with this method, a survival percentage of about 70 percent of the grafts attempted was achieved. This method was as follows: The root stock consisted of a bolting sugar beet with a seed stalk from six to fifteen inches in height. One of the leaves and the axillary bud was cut away on the seed stalk in a very succulent area (Figure 1) below the area of elongation and above the hard tissue of the lower seed stalk. A longitudinal incision, from one-fourth to three-eighths of an inch long and extending about three-fourths of the way through the stem, was made in the area of the excised leaf and bud with a razor blade or other sharp instrument (Figure 2). The scion consisted of a small seedling from the cotyledon to the four-leaf stage of growth.

At about the level of the soil, either a slanting or wedge shaped cut was made through the hypocotyl of the scion, so the root was completely cut off. This scion was then placed into the incision of the seed stalk (Figure 3), care being taken to match one of the cut surfaces of the scion with the side of the incision on the root stock. This graft was then bound into place with a piece of twine (Figures 4 and 5). All other leaves and buds were removed from the seed stalk except one leaf above the graft. This one leaf is left to prevent the seed stalk from dying back below the graft before the tissues have grown together. The completed graft was then placed in a cool, shaded area in the greenhouse and left until signs of growth began to show in the scion. This usually required about two weeks. When they began to show growth, they were placed out in the sunlight in the greenhouse where they continued to develop vigorously and many of them flowered very readily. The twine should be cut soon after growth starts.

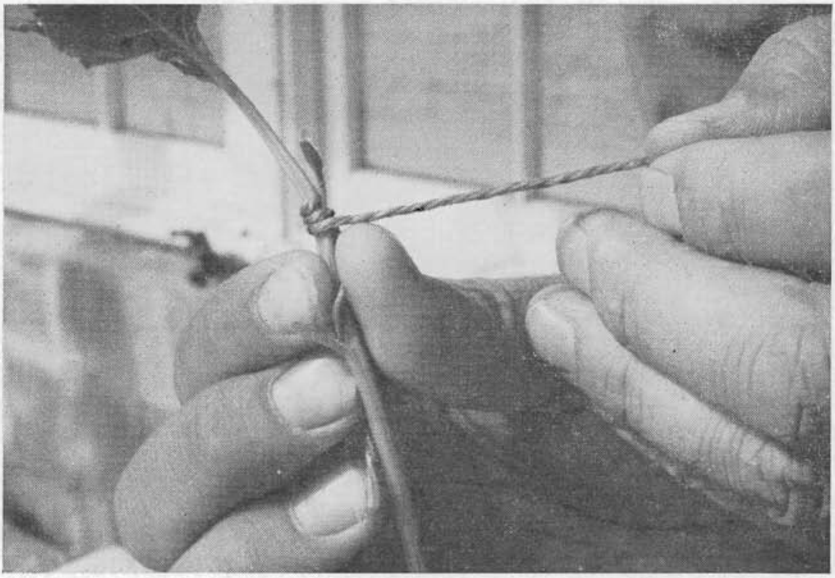

Figure 4.—Twine is wrapped firmly around graft above and below the scion to prevent the sides of the incision from healing away from the scion.

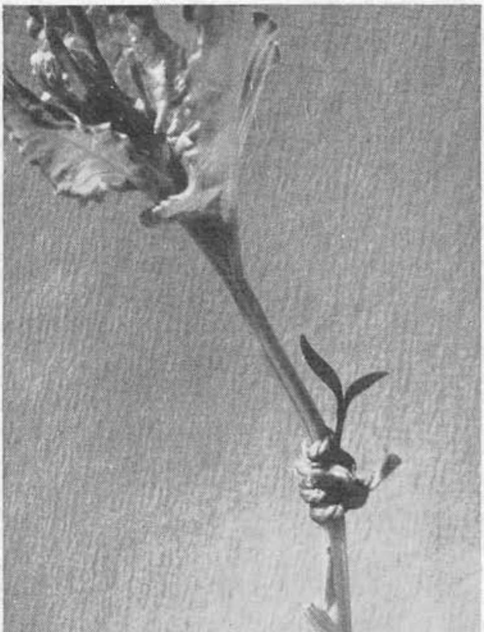

Figure 5.—The completed graft is placed in a cool, shaded section of the greenhouse until the scion begins to show signs of growth. When scion begins to grow, the twine should be cut to prevent binding. Use of rubber bands for tying grafts may be better than twine.

to keep from restricting growth. At first, there appeared to be a significant increase in survival by refrigerating the completed graft at 45° F. for twenty-four to forty-eight hours. A later comparison failed to confirm this advantage, so the refrigeration was discontinued in later grafting work.

By using this method, sugar beet seedlings and hybrids between sugar beets and members of the section, *Patellares,* were satisfactorily grafted onto sugar beet seed stalks. Similar seedlings, however, did not survive when grafted onto plants of *B. patellaris, B. procumbens,* or *B. webbiana,* nor did seedlings of these latter three species survive when attempts were made to graft them onto sugar beet seed stalks, although only small numbers of plants were involved in these last mentioned types of grafts. Axillary buds of other sugar beets were readily grafted in this manner and could be used to maintain clones of self-sterile sugar beets if so desired.

An annual beet was the source of the seed stalk most often used. This was because of the ease with which a seed stalk of the desired size could be obtained. Biennial beets were used with equal success when available.

The F_1 hybrids which continued to grow after they had been grafted exhibited a wide range of appearances. As others have observed, none of the plants completely resembled either the sugar beet or the wild parent, but almost all degrees of intermediate expression for most visible characteristics between the two parents could be found in the hybrids. This range was typical of leaf type and plant type. All hybrid plants bolted but only about 60 percent of them flowered. Pollen sterility appeared to be complete, as all anthers were entirely colorless. When supplied with an abundance of sugar beet pollen, 40 percent of the flowering plants did produce some normal appearing seed. This seed more closely resembled the seed of the wild parent than it did the sugar beet, in that it was borne in clusters of three which readily separated into single locule seeds upon ripening. The percentage of florets setting seed varied from less than one in some plants to over fifty in others. The viability of this seed, however, was extremely low. From approximately five-hundred seeds produced, only eight seedlings grew. Unfortunately, these, like the F_1 hybrids, would not grow to maturity on their own roots and required grafting for survival. The roots of these backcrossed plants, however, did show considerably more development than did the roots of the F_1 plants. In most of the backcrossed plants there had been some enlargement of the primary root and some development of secondary roots in well defined root zones. These secondary roots, however, became necrotic and to propagate the plants, they were grafted. All grafts attempted in these backcrossed plants were successful.

In appearance, these backcrossed plants were almost indistinguishable from sugar beets. The leaf shape was almost the same. Bolters in these backcrossed plants showed about the same degree of apical dominance that is shown by the sugar beet and there was no suggestion of a viny type of growth demonstrated by the wild parents. At this time there are several of these plants flowering but it is not known whether they will produce seed. In this generation there are appearing plants with very yellow anthers, however, it has not been possible to dissect pollen from them.

There has been a large amount of work done in recent years in California in the development of very slow bolting varieties. As the varieties become more and more slow bolting, it becomes increasingly difficult to get complete participation and exact reproduction of these varieties in the seed fields. It appears that a point will be reached where the improvement by artificial selection for slow bolting will just about be nullified by the natural selection pressure in the seed fields toward the reproduction of the easier bolting segments of the varieties (3). Much is known as to the conditions required to induce bolting or seed stalk production; but almost nothing is known as to the nature or the action of the material that stimulates a sugar beet plant to change from the vegetative to the reproductive cycle. When this grafting technique became available, it offered a method by which one slight phase of this problem could be studied. Because the root stock is in the reproductive phase at the time the graft is made, it must possess sufficient of the stimulant material to induce and promote seed stalk development. An attempt was made to determine if this material, whatever it might be, would be transferred across the graft union and in what amounts. If the stimulus to produce a seed stalk was readily transferred across the graft union, it would provide a way of hastening the flowering process in extremely slow bolting breeding material that, at present, is extremely difficult to induce into flowering.

Three strains of sugar beets were selected on the basis of their bolting tendencies. They were classified as easy bolting, intermediate bolting, and very slow bolting. Seedlings of each were grafted onto a separate seed stalk in the manner described above. Six successful grafts were made of each class. They were kept in a warm greenhouse under conditions of natural day length. Under these conditions, normal biennial beets will not produce seed stalks. After about eight weeks, seed stalks began to appear in the grafts of the easy bolting strain and finally five out of the six grafts in that class produced a seed stalk. None of the grafts of the intermediate or very slow bolting strains showed any indications of developing a seed stalk. It seemed apparent then, that some of the stimulus for the development of a seed stalk is transferred across the graft union, but not in sufficient quantities to stimulate seed stalk production in sugar beet types that would be considered slow bolting.

While it appears that the three *Beta* species of the section, *Patellares,* are resistant to the attacks of the sugar beet nematode, almost nothing is known of the nature of this resistance. As previously pointed out, Gaskill has reported that F_1 hybrids between swiss chard and these wild species have shown resistance to the sugar beet nematode, while the swiss chard parent is susceptible. This could indicate that resistance is dominant. It is not known whether the substance or condition responsible for this resistance is a characteristic of the root itself or due to something manufactured in the leaves and subsequently translocated to the root. Assuming Gaskill's findings applied also to the crosses under discussion here, different graft combinations offered a method of casting some light on this problem. Because all grafts were made onto sugar beet plants, if nematode resistance or susceptibility was due to a characteristic of the root itself, it would make no difference whether sugar beet seedlings or the supposedly resistant F_1 seedlings were used for the scion, all plants would be susceptible. If, however, resistance was due to some substance manufactured in the leaves and translocated into the root, there should be a difference in nematode resistance depending upon whether a sugar beet or F_1 hybrid seedling was used as a scion. Mr. George Wheatley, of the Sugar Section, Agricultural Research Service, U. S. Dept. of Agriculture, who is working on the sugar beet nematode problem consented to make the comparison between these two types of plants under heavily infested, controlled conditions in the greenhouse. At the end of his tests he could not determine any differences between the roots that had sugar beets grafted onto them and those that had F_1 hybrids for grafts. This would indicate that probably nematode resistance is a characteristic of the root itself.

Summary

A method of grafting is described by which a relatively high percentage of normally seedling lethal plants of inter-specific hybrids within the genus, *Beta*, can be propagated.

The F_1 plants of these interspecific crosses showed a wide range of variability between the two parents for most all visible characteristics; however, when these F_1 hybrids were backcrossed to sugar beet plants, the resulting progeny were almost indistinguishable from the sugar beet, except that they would still not grow on their own root and needed grafting for survival.

While the method of grafting was used here primarily to prolong the lives of F_1 hybrids and their backcrossed progeny, the method could have use in propagating clones of self-sterile sugar beets if so desired.

It was shown that some of the material associated with the stimulus that promotes the development of a seed stalk in sugar beets was transferred across the graft union, but not in sufficient quantities to cause slow bolting strains to bolt.

Indications were that the nature of the resistance to the sugar beet nematode is a characteristic of the root itself and not something that is manufactured in the leaves and later translocated into the root.

References

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