Table 1. Effect of cetyl alcohol and sodium fluoride on biochemical changes in potato tubers (recorded 3 months after storage at a temperature range of 29° to 35°C).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Chemical</th>
<th>Rots (%)</th>
<th>Total soluble solid (%)</th>
<th>Ascorbic acid content (mg/100g)</th>
<th>Starch content (%)</th>
<th>Protein content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>58.3</td>
<td>5.0</td>
<td>4.22</td>
<td>9.95</td>
<td>1.74</td>
</tr>
<tr>
<td>Cetyl alcohol</td>
<td>10</td>
<td>31.6*</td>
<td>4.0*</td>
<td>7.39*</td>
<td>12.48*</td>
<td>1.68</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>48.8*</td>
<td>4.5*</td>
<td>7.03*</td>
<td>10.67*</td>
<td>1.72</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>58.4</td>
<td>5.0</td>
<td>5.63*</td>
<td>10.09</td>
<td>1.74</td>
</tr>
<tr>
<td>Sodium fluoride</td>
<td>10</td>
<td>59.9</td>
<td>5.0</td>
<td>5.28</td>
<td>8.50</td>
<td>1.77</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>55.1</td>
<td>5.0</td>
<td>5.28*</td>
<td>8.60</td>
<td>1.76</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>52.3</td>
<td>5.0</td>
<td>4.22</td>
<td>8.73</td>
<td>1.75</td>
</tr>
</tbody>
</table>

*Significantly different from control at 5% level.

each treatment, 2 kg of tubers were dipped for 1 hr in 2 liters of an aqueous solution of the desired concentration. Tubers dipped in distilled water served as the control. There were a total of 7 treatments which were replicated 3 times following a completely randomized design. The tubers were stored for 3 months in the laboratory to study the effect of treatments on rotting and qualitative changes in tubers during this period. During the day (6 AM to 6 PM) there was diffused light in the laboratory; at night it remained dark. The average temperature of the laboratory during this period varied from 29° to 35°C.

Percentage of tuber rot on a weight basis was determined at 14 day intervals. Total soluble solids (TSS) content of tubers were determined before treatment and 3 months after storage by a hand refractometer. Ascorbic acid was determined by the iodine titration method (5); starch by direct acid hydrolysis, and protein content by Kjeldahl’s method of tubers before and 3 months after storage.

Rotting was reduced by cetyl alcohol at 10 and 25 mg/liter (Table 1) due to the action of this chemical in coating the tuber lenticels checking the entrance of microorganisms (7). This is comparable to its effect on stomata and cuticle reported in leaves (2). Cetyl alcohol at 50 mg/liter was ineffective in reducing rots, possibly due to toxicity (3). Sodium fluoride treatments were ineffective in controlling rots.

TSS of tubers was less than the control in treatments with 10 and 25 mg/liter cetyl alcohol. Control tubers lost water as a result of transpiration and the TSS increased. This was not the case in tubers treated with the antitranspirant as the water loss resulting from transpiration was controlled (2). Tubers treated with sodium fluoride had the same percent TSS as the control.

The ascorbic acid content was highest in tubers treated with 10 mg/liter cetyl alcohol followed by 25 mg/liter. At the highest concentration, the ascorbic acid content decreased but was still higher than 50 mg/liter sodium fluoride and the control. Tubers treated with 10 and 25 mg/liter sodium fluoride contained more ascorbic acid than the control. As cetyl alcohol coats the lenticels of tubers and controls diffusion of oxygen through them (4), the oxidation of ascorbic acid is minimized. Sodium fluoride acts by inhibiting oxygen uptake directly (1).

Starch content of tubers treated with cetyl alcohol increased significantly and was 12.5% with the 10 mg/liter treatment. At higher concentrations the starch content decreased gradually, but still the values were more than the treatments with sodium fluoride and the control. Cetyl alcohol increases water content of tubers by checking transpiration and this high water content of tubers does not favor hydrolysis of starch to sugar (8).

Tubers treated with sodium fluoride contained less starch than the control.

# Interspecific Hybridization in Euphaseolus through Embryo Rescue

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Additional index words. embryo culture, Phaseolus vulgaris, Phaseolus coccineus, Phaseolus acutifolius, Phaseolus lunatus

Abstract. About 2000 pollinations of Phaseolus coccineus x P. acutifolius, P. coccineus x P. vulgaris, P. vulgaris x P. acutifolius, and F1, (P. vulgaris x P. coccineus) x P. acutifolius produced more than 1500 excisable embryos, about half of which elongated and produced leaves and roots when embryo cultured on a relatively simple medium in the dark. Damaging cotyledonary buds on small embryos or removing cotyledons from larger embryos before culture enhanced germination. Transplanting germinated embryos into pots resulted in greater than 50% mortality. Hybrids tended to be intermediate to parental species in vegetative and floral characteristics but percent stainable pollen was lower. Anthers did not dehisce and percent stainable pollen was lowest in P. vulgaris x P. acutifolius and F1, (P. vulgaris x P. coccineus) x P. acutifolius hybrids. Since P. coccineus x P. acutifolius and P. coccineus x P. vulgaris hybrids were sufficiently fertile to produce advanced generations, P. coccineus may be useful as a bridge between P. vulgaris and P. acutifolius.

Interspecific hybridization within section Euphaseolus of the genus Phaseolus has been of interest to bean breeders (1, 2, 3, 5, 9, 10, 13). It has been suggested

Literature Cited

that incorporation of 3 species in Eupha-
seolus, P. vulgaris, P. coccineus, and P. acutifolius, into a common gene pool would enable plant breeders to select beans of the P. vulgaris type with drought tolerance and resistance to a wide range of diseases (5, 13). However, distinct bar-
riers to interspecific hybridization exist be-
tween P. vulgaris and most other spe-
cies (1, 12). Causes for failure to obtain
useful interspecific hybrid beans can be
grouped into 4 categories: 1) fertilization
failure due to restricted pollen tube
growth or function, 2) lack of normal seed
development, 3) abnormal growth
patterns in hybrid plants, and 4) sexual
sterility in hybrids (1, 2). A major diffi-
culty in obtaining interspecific hybrids in
Euphaesolus appears to be lack of normal
seed development, since fertilization oc-
curs but embryos abort 8-24 days after
pollination (9, 14, 16). Therefore, we
resorted to embryo culture, hoping to de-
velop a means of producing large num-
bers of interspecific hybrids within
Euphaesolus.

The species used were P. acutifolius, P. coccineus, P. lunatus, and P. vulgaris. Plants were grown and pollinated in a greenhouse maintained at 27/22°C (day/ night). Emasculation and pollination
techniques were as described by Bui-
shand (4). To determine whether restrict-
ed pollen tube function occurred in our
material, reciprocal interspecific pollina-
tions were made in all combinations and
intraspecific pollinations were made for
comparison. The pistils were harvested
24 hr after pollination, fixed for 24 hr in
formalin acetic acid (FAA), then rinsed
30 min in distilled water, and softened 20
hr in 8 N NaOH at room temperature.
The pistils were rinsed again, stained for
8 hr in 0.1% aniline blue in 0.1 M K3PO4,
and squashed in 2 glycine:1 water. Ob-
servations were made with a blue-light
fluorescent microscope. No difference
between interspecific and intraspecific
pollen tube growth was detected. All pol-
linated pistils exhibited abundant pollen
tubes at the base of the ovary 24 hr after
pollination. These data agree with the
findings of other workers (7, 9, 14, 16).

Pods from interspecific pollinations were
harvested 15-20 days after pollina-
tion and surface sterilized by submerging
for 2 min in 95% ethanol, then 20 min in
0.5% sodium hypochlorite, followed by
rinsing in sterile distilled water. Embryos
were excised aseptically and cultured in 8
dram vials on slants of the Emsweller et
al. medium (6) with 4% sucrose. Vials
were incubated in the dark at 25°C until
the embryos began elongating and form-
ing leaves. Occasionally one or both co-
tyledonary buds were damaged in excis-
ing the embryos. We observed that
cotyledons did not develop in culture
when cotyledonary buds were damaged, and more importantly, embryos without
cotyledons elongated and formed true
leaves faster than intact embryos. Un-
damaged embryos often produced nor-
mal-sized cotyledons in culture, then
cess development and died. Therefore,
we routinely damaged both cotyledonary
buds on small (heart-shaped) embryos or
removed the cotyledons from larger em-
broys before placing them on the culture
medium.

When embryos elongated and pro-
duced at least one true leaf, vials were
placed in Cool-White fluorescent light
(160 {eq}\mu\text{E m}^{-2} \text{ sec}^{-1} {/eq}) for 16 hr each day at
22°C. After 5-10 days, embryos with at
least 1 expanded green leaf were re-
moved from the culture vials, washed in
distilled water, and planted one per 5 cm
plastic pot. Pots were covered with plastic
bags to maintain high relative humidity
and returned to the environment de-
scribed above. Initially, we used fine ver-
miculite as the potting medium but only
7% of the seedlings survived. Therefore,
we tested several potting media. About
30% of the 250 seedlings transplanted in-
to a mixture of 1 fine vermiculite:1 milled
sphagnum moss survived, 37% of the 237
transferred into 1 coarse vermiculite:1 milled
sphagnum survived, and 40% of the
295 placed in 2 coarse vermiculite:1 milled
sphagnum:1 steamed soil survived (Table 1).

Using P. lunatus as the female in
crosses with P. vulgaris and P. coccineus
resulted in early pod abscission. Ovules
were shrivelled and embryos were under-
developed and not excisable. This is in
agreement with data reported by Mok et
al. (12). The cross P. vulgaris P. lunatus
usually resulted in pod set, but we found
no excisable embryos.

To determine percent stainable pollen,
mature anthers were collected from each
putative hybrid prior to dehiscence, al-
lowed to dehisce on a glass slide or teased
open if indehiscent, and the pollen
stained with acetocarmine. We felt that
pollen stainability, as an estimate of fer-
tility, would be an excellent criterion for
verification of interspecific hybridity.
Contamination from self or intraspecific
pollen should not produce plants with re-
duced fertility. Plants used for interspec-
cific crosses exhibited 95 to near 100%
stable pollen. Only 2 plants from cul-
tured embryos appeared to be non-hy-
bred, these from P. coccineus x P. acutifoli-
us pollinations. They were of P. coccineus
phenotype, averaged nearly 100% stable
pollen, and therefore were excluded from our data. In another
attempt to verify hybridity, we looked at
meiosis in putative P. coccineus x P. acuti-
folius and P. vulgaris x P. acutifolius hy-
breds. Young inflorescences were fixed in
2 absolute ethanol:1 propionic acid satu-
rated with ferric acetate (15). The fixative
was replaced with 70% ethanol for stor-
age. Anthers were squashed in acetocar-
mine. Pairing was irregular and bridges
accompanied with laggards were ob-
served in all hybrids examined.

Hybrids tended toward intermediacy in
vegetative characters. Exceptions were
abnormal plants, dwarfed, with curled
leaves and apparent virus symptoms,
which often occurred among P. vulgaris x
P. coccineus and P. vulgaris x P. acutifi-
olius progenies. These plants fit the descrip-
tion of cripple types reported by Smartt
(14) and, although occasionally surviving
transplanting, none grew to flowering.
Other workers have also reported abnor-
malities in interspecific bean hybrids (11,
12, 16). Normal hybrids from P. vulgaris x

Table 1. Pod set as a function of number of pollinations, embryos transplanted to pots as a function of the
number of embryos excised, surviving plants as a function of the number transplanted from culture, and
mean percent stainable pollen in plants resulting from interspecific pollination in Euphaesolus.

<table>
<thead>
<tr>
<th>Pollination</th>
<th>Pod set (%)</th>
<th>Cultured embryos transplanted (%)</th>
<th>Transplanted embryos surviving (%)</th>
<th>Mean stainable pollen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. coccineus x P. acutifolius</td>
<td>10</td>
<td>25</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>P. coccineus x P. vulgaris</td>
<td>50</td>
<td>20</td>
<td>33</td>
<td>66</td>
</tr>
<tr>
<td>P. vulgaris x P. coccineus</td>
<td>90</td>
<td>20</td>
<td></td>
<td>36</td>
</tr>
<tr>
<td>P. vulgaris x P. acutifolius</td>
<td>80</td>
<td>80</td>
<td>40</td>
<td>5*</td>
</tr>
<tr>
<td>F1 (P. vulgaris x P. coccineus) x P. acutifolius</td>
<td>22</td>
<td>53</td>
<td>14</td>
<td>14*</td>
</tr>
</tbody>
</table>

*This pollination produced normal seeds, thus embryos were not cultured.
†Anthers indehiscent.
P. coccineus had deep pink flowers unlike the bright red flowers of the male parent and were sufficiently fertile to produce pods with normal seeds after backcross, self, or inter-hybrid pollination. The reciprocal, P. coccineus × P. vulgaris, also produced some cripple types. Flower color was closer to the red of P. coccineus and, as reported by Ibrahim and Coyne (11), and fertility was higher. Self pollination of the interspecific F₁ produced an F₂ which segregated for flower color, producing some plants with deep scarlet to purple flowers of a size characteristic of P. coccineus. P. coccineus × P. acutifolius hybrids bore pink to red-pink flowers resembling those of the female parent. As in P. coccineus, the stigmatic beard prevented automatic self pollination. Cuttings of these hybrids planted in the field produced pods following bee activity. However, embryos excised from the pods were lost after transplanting into pots. Embryos produced by the cross F₁ (P. vulgaris × P. coccineus) × P. acutifolius were larger than those from 2-species crosses when excised and grew more rapidly in embryo culture. However, the fertility of the 3-species hybrids was low and the anthers did not dehisce (Table 1). The number of interspecific hybrids recovered using embryo culture far exceeded our expectation and the degree of success previously reported (3, 8, 12). Removal of cotyledons and embryo culture in the dark may have been responsible. Additional work toward improving survival after transplanting from culture and increasing fertility in the resulting hybrid seedlings is warranted. Since P. coccineus × P. vulgaris and P. coccineus × P. acutifolius hybrids seem fertile enough to produce advanced generations, the use of P. coccineus as a bridge between P. vulgaris and P. acutifolius may result in 3-species hybrids with useful fertility.

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**Root Morphological Characteristics of Kidney Beans as Influenced by Within-row Spacing**

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**Additional index words.** Phaseolus vulgaris, uprooting, resistance, adventitious roots, basal roots, taproot

**Abstract.** Root morphological characteristics of ‘Redkote’ and ‘Redkloud’ kidney bean plants (Phaseolus vulgaris L.) were measured during 2 growth stages at 5, 10, 15, and 20 cm within-row spacings under field conditions. Significantly higher total root weight, shoot weight, basal root weight, and stem and hypocotyl diameters of individual plants occurred as within-row spacing increased. Uprooting resistance, taproot weight, and taproot diameter increased as within-row spacing increased up to 15 cm followed by a nonsignificant increase at 20 cm. No differences in adventitious root weight or shoot:root ratios occurred among within-row spacing treatments.

‘Redkote’ root parameters were significantly higher than those of ‘Redkloud’, with the exception of adventitious root weight and uprooting resistance. Seed yields were highest for 15 cm spacing although not significantly more than 5 cm spacing. All parameters with the exception of basal root number were significantly lower during anthesis when compared with the full population growth stage.

Tanaka (8) has classified legume crops into alfalfa, vetch, and intermediate (soybean) root developmental types. Root growth of 26 legume crops differed in their ability to elongate, branch, and thicken (9). Increasing root size of many field crops has been reported to improve water and nutrient uptake, lodging resistance, and ultimately yields. Stoffella et al. (6) reported that plants of 4 black bean lines having a large root size were more upright and yielded higher than 2 cultivars (‘Black Turtle Soup’ and ‘Strain 39’). The 4 lines had significantly greater basal root (roots arising from the basal section of the hypocotyl) weights and diameters and required more uprooting force than the 2 cultivars, suggesting that basal roots were responsible for lodging resistance.

Hidalgo (2) observed that a large sized main central root and a large number of secondary roots were able to extract greater amounts of water than smaller roots in dry beans. Johnson (3) reported that ‘Redkloud’, with a double grafted root system had a lower shoot and root water potential than a single root system under water stress. However root water potential was higher for the double root system under control conditions, suggesting that a larger root system may be advantageous where water stress is lacking.

Information on root morphological characteristics of kidney beans is limited. The purpose of this investigation was to measure several root morphological characteristics of kidney beans as influenced by within-row spacings.

Two red kidney bean cultivars (‘Redkote’ and ‘Redkloud’) were grown under field conditions during summer 1978, at