Hybrid Weakness in *Phaseolus vulgaris* L. I. Disruption of Development and Hormonal Allocation

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Abstract. A reduced concentration of cytokinins may cause the abnormal growth and development found in F1 hybrids between Andean and Mesoamerican races of *Phaseolus vulgaris* L. In this study, concentrations of the transportable cytokinin zeatin riboside (ZR) were measured by ELISA for ZR (cross reactivities dihydrozeatin, 14%, zeatin 7.6%) in roots, stems, and leaves of a *Phaseolus* Mesoamerican landrace (*P. vulgaris* L. cv. Redkloud), an Andean landrace (*P. vulgaris* L. cv. Batt), and their F1 hybrids. Concentrations of ZR in roots and leaves of F1 hybrids were significantly less than that found in roots and leaves of parental cultivars. Approximately 90% of the ZR found in F1 hybrids was found sequestered in the stems, whereas cytokinins of the parental cultivars were distributed throughout the plant (roots: Batt 37%, Redkloud, 44%; stems: Batt 35%, Redkloud 42%; leaves: Batt 28%, Redkloud 14%). These results suggest that abnormal growth and development of F1 hybrids may involve interruption of the regulation of cytokinin allocation, thereby disrupting the root-shoot feedback loop between root-sourced cytokinins and putative shoot-produced factors.

Key Words. Cytokinin—Zeatin riboside—Hormone—*Phaseolus*—Root-shoot interactions——DL genes—Adventitious roots

Breeders have long been interested in barriers to hybridization, and the results of their studies have produced interesting systems that can be used by researchers to study fundamental physiology. In *Phaseolus vulgaris*, specific hybrids between parents of Mesoamerican and Andean origin exhibit root and shoot abnormalities (Gepts and Bliss 1985, Shii et al. 1980, 1981, Singh et al. 1988). Symptoms include reduction in root and shoot mass, adventitious root growth on stems, leaf chlorosis, early senescence, and eventual shoot death (Singh and Gutierrez 1984). This condition, called hybrid weakness (or dwarf lethality), is usually lethal and appears to be controlled by two semidominant, complementary alleles referred to as dosage lethal (DL), one expressed in the root (DL1) and one expressed in the shoot (DL2). F1 hybrids homozygous at both DL loci are lethal. Individuals that are homozygous dominant at one locus and heterozygous dominant at the other locus are sublethal. Homozygous recessives at one or both loci show no symptoms related to hybrid lethality.

Although the specific causes of hybrid weakness are unclear, it appears that cytokinins may be involved (Shii et al. 1980, 1981). Roots have been found to be rich in cytokinins and are believed to supply them to shoots, which in turn can affect leaf growth (Henson and Wareing, 1976, Loeffler and Overbeek 1964, Neuman et al. 1990, Weiss and Vaadia 1965, Went 1938, Went and Bonner 1943). Several cytokinins have been identified in xylem sap (Hall et al. 1987, Palmer and Wong 1985). Interruption of the xylem root-shoot connection, as well as stress in the root zone, has been found to decrease xylem sap cytokinin concentrations and increase leaf senescence (Itai and Vaadia 1965). Although leaves also produce cytokinins, these appear to be storage forms, therefore root-produced cytokinins may have an important role in shoot development (Singh et al. 1988, Went 1938). In this study, we have characterized growth characteristics and hormonal status of roots, stems, and leaves of parental and F1 crosses carrying the alleles for hybrid weakness to begin to understand mechanisms related to the integration of root and shoot growth. Although there are many metabolic forms of the cytokinins, this study is focused on zeatin riboside (ZR), the cyto-

kinin most often associated with transport. ZR is found in xylem sap and has been shown to increase the growth of leaf discs *in vitro* (Neuman et al. 1990). Because ZR may have a role in the integration between roots, shoots, and leaf growth, we hypothesized that ZR concentrations would be reduced in roots and shoots of the F1 hybrids. We found that although ZR concentrations are reduced in roots and leaves of the F1 hybrids, stems have very high concentrations of ZR, suggesting that there is a break-down in the transport or allocation of this cytokinin instead of a deficiency of cytokinins.

Materials and Methods

Plants

Seeds of parents and F1 hybrids of common bean (*P. vulgaris* L, cv Redkloud; *P. vulgaris* L, cv. Batt; *P. vulgaris* L, cv. Redkloud × cv. Batt) were grown in 6-inch pots, one plant per pot, in a controlled room at 30–35 °C for a 16 h photoperiod, $300\mu E\ m^{-2}s^{-1}$.

Growth Analysis

For growth analysis after development of trifoliate leaves, growth of roots, stems and leaves was determined from weekly measurements of three replications over a 4-week period. Leaf area was determined using the LICOR Li-100. Root growth was quantified by counting the number of root tips. Plant material was dried for 3 days in a 60°C oven and weighed. All experiments were repeated at least two times.

Hormone Analysis

For hormone analysis, three replications of plant material harvested weekly over a 4-week period were frozen in liquid nitrogen and stored at −70 °C. Frozen material was ground in liquid nitrogen, extracted in ice-cold HPLC grade methanol buffer, agitated for 30 min, and centrifuged at 2,500 rpm for 10 min. Supernatant was evaporated, dried, and partially purified as previously described (Neuman et al. 1990). Extracts were dried and resuspended in 0.01 M phosphate buffer (pH 7.4).

Zeatin riboside concentrations were measured in young, growing roots, stems, and leaves by ELISA as previously described (Neuman et al. 1990). The methodology and cross reactivities of the antisera for ZR were previously reported (Smit et al. 1990). Because there are appreciable cross reactivities of the ZR antiserum with dihydrozeatin (DHZ, 14.1%) and zeatin (Z, 7.6%), cross reactants could contribute to the total estimated concentration of the cytokinin of interest. Therefore all data reflect the quantity of zeatin riboside plus any immunological equivalents. [3H]-DHZ (approximately 2,500 dpm, 30–60 Ci/mmol; Amersham, UK) was added to all samples to check losses by isotope recovery. Recoveries varied between 40–80%. Although samples were corrected for recovery, no corrections were made for added [3H]-DHZ because the ratio of tritium-labeled DHZ to endogenous concentrations within plant tissues was very small and much less than the sensitivity of the assay (0.01 pmol).

Results

Growth Analysis

Root and shoot growth of F1 hybrid plants of *P. vulgaris* did not progress compared with parental cultivars (Fig. 1). No differences were found in the development of leaf
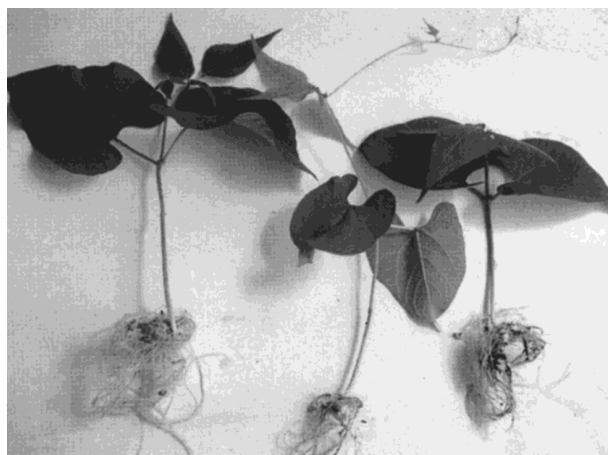

Fig. 1. The F1 hybrid *(center)* exhibits stunted root and shoot growth, chlorotic leaves, and adventitious root development in the stems compared with normal growth in Batt *(left)* and Redkloud *(right)*.

area between the parental cultivars, Batt and Redkloud, over the 4-week period (Fig. 2). Leaf area of the F1 hybrid did not increase significantly after the second week, resulting in very small leaves compared with parental cultivars.

Dry mass of the F1 hybrid was greatly reduced by the third week when compared with parental cultivars (Fig. 3). In addition, leaves of F1 hybrids became chlorotic around the second week. Dry mass accumulation in stems was also reduced in F1 hybrids, although not until the third week. By the fourth week, stems of F1 hybrids appeared woody compared with those of the parental cultivars, and adventitious roots began to appear on these stems. Root dry mass was significantly reduced by the second week in F1 hybrids compared with the parental cultivars (Fig 3). Interestingly, reduction of stem and leaf dry mass in F1 hybrids was not significantly different until the third week.

In addition to decreased root dry mass, the number of root tips in F1 hybrids was reduced compared with those of parental cultivars (Fig. 4). By the end of the first week, the number of root tips in F1 hybrid plants was reduced by 60% compared with that of parental cultivars. By the end of the experiment, both Redkloud and Batt had more than 500 root tips per plant, whereas the F1 hybrid had approximately 100 root tips per plant.

Hormonal Analysis

In addition to effects on growth and development, the cytokinin status of F1 hybrids was unlike that of parental cultivars. By the fourth week, leaf concentrations of ZR-type cytokinins (henceforth referred to as ZR) in F1 hybrids was decreased compared with parental cultivars
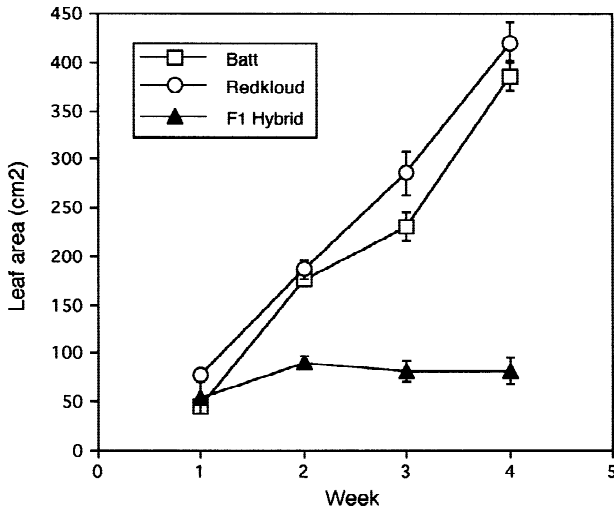
Fig. 2. Changes in leaf area during three weeks of growth in Batt, Redkloud, and F1 hybrids.

(Fig. 5). By the fourth week, ZR concentrations in F1 hybrids increased at least twofold over the concentrations measured in stems of parental cultivars. In roots, ZR concentrations of F1 hybrids were similar to that of Batt, whereas Redkloud had increased concentrations of ZR. By the fourth week of the experiment, the concentrations of ZR measured in roots of parental cultivars were similar (approximately 60 pmol g^{-1}), whereas the ZR concentrations in roots of F1 hybrids were significantly lower (approximately 8 pmol g^{-1}). Interestingly, although the concentrations of ZR-type cytokinins were altered in individual organs of the plant, the total plant concentration of cytokinins in parental cultivars and the F1 hybrids were similar because of the high concentrations found in the stems of the F1 hybrids (Fig. 6). Our results indicate that most ZR-cytokinins in F1 hybrid plants were being allocated to stems, as opposed to parental cultivars in which ZR-cytokinins were more equally distributed among leaves, stems, and roots.

Discussion

Researchers have long recognized that a balance exists between the growth of roots and shoots. To address questions about how roots and shoots are integrated, we used a developmentally disabled hybrid of *P. vulgaris* carrying semidominant genes of Mesoamerican and Andean origin. As previously reported by Shii et al. (1980, 1981), the F1 hybrid is characterized by reduced root and shoot growth. In addition, F1 hybrids rarely make seed, especially when grown at elevated temperatures. On the basis of feeding and grafting experiments, Shii et al. (1980, 1981) suggested that one of the causal agents for hybrid
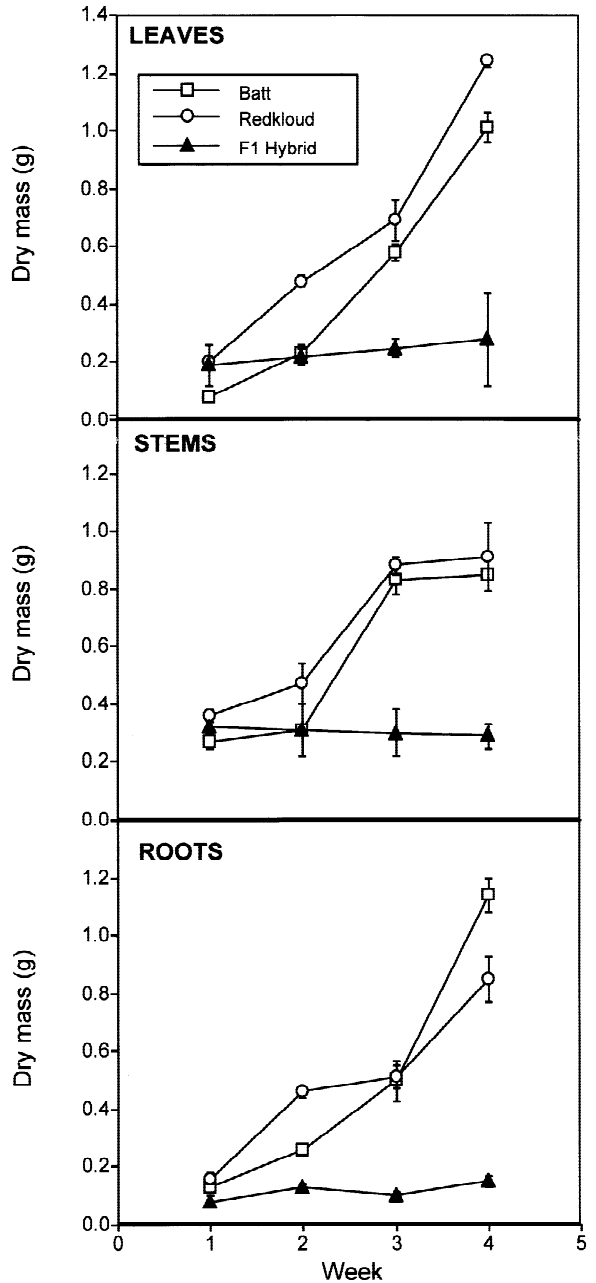

Fig. 3. Changes in dry mass during 4 weeks of growth in Batt, Redkloud, and F1 hybrids.

weakness of these F1 hybrids could be a disruption of cytokinin metabolism. The data presented here support that conclusion and provide additional evidence for the important role of hormones in the integration between roots and shoots.

Although the concept of integration by hormones moving between roots and shoots is not new (Davies et al. 1987, Incoll and Jewer 1987, Kulaeva et al. 1996), the identification of DL plants with disturbed root-shoot re-
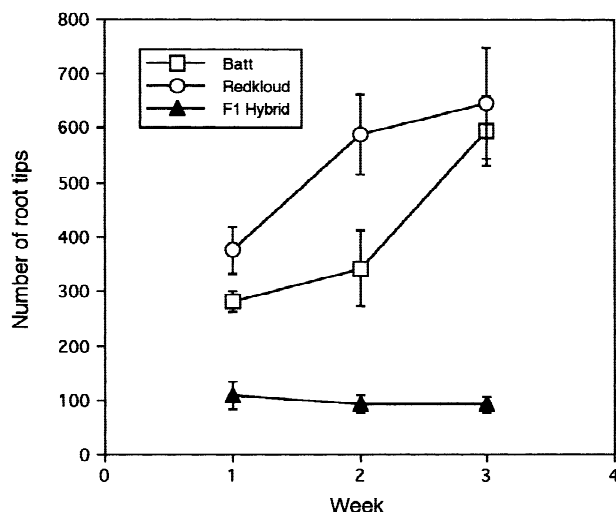
Fig. 4. Changes in number of root tips during 3 weeks of growth in Batt, Redkloud, and F1 hybrids.

lations provides a novel experimental system with which we can test hypotheses concerning hormonal reduction of root-shoot integration. In this study, it has been demonstrated that reduced growth of F1 hybrids is associated with changes in the allocation of ZR-type cytokinins between roots and shoots. The most interesting finding of this study is that although the concentration of ZR-type cytokinins is decreased in roots and leaves of F1 hybrids, the amount of cytokinins in the whole plant did not decrease. Instead, most of the cytokinins appear to be localized in the stems in the F1 hybrids. Therefore, although a genetically caused underproduction of root cytokinins may exist, this is not the only factor involved in cytokinin disruption in this hybrid. Our results therefore suggest that cytokinin allocation may play an important role in development in this species.

The specific role of cytokinins in root growth remains unresolved. In some species, exogenous cytokinins have been shown to inhibit root growth (Auer 1996, Martin et al. 1997). In other species such as bean, cytokinins have been shown to enhance root growth (Shii et al. 1980). It has also been reported that cytokinin-like compounds increase during adventitious root formation in stem cuttings, suggesting that cytokinins may have a role in the early stages of root growth (Taylor and van Staden 1997). In this study, the development of adventitious roots in stems occurred in conjunction with increased concentrations of cytokinins. Whether the high concentration of cytokinins in these stems is related to root growth cannot be determined from the results presented here. Shii et al. (1980, 1981) found that applications of benzylaminopurine (BAP), a synthetic cytokinin, enhanced the growth of the primary root system of Mesoamerican/Andean hybrids similar to the F1 hybrids
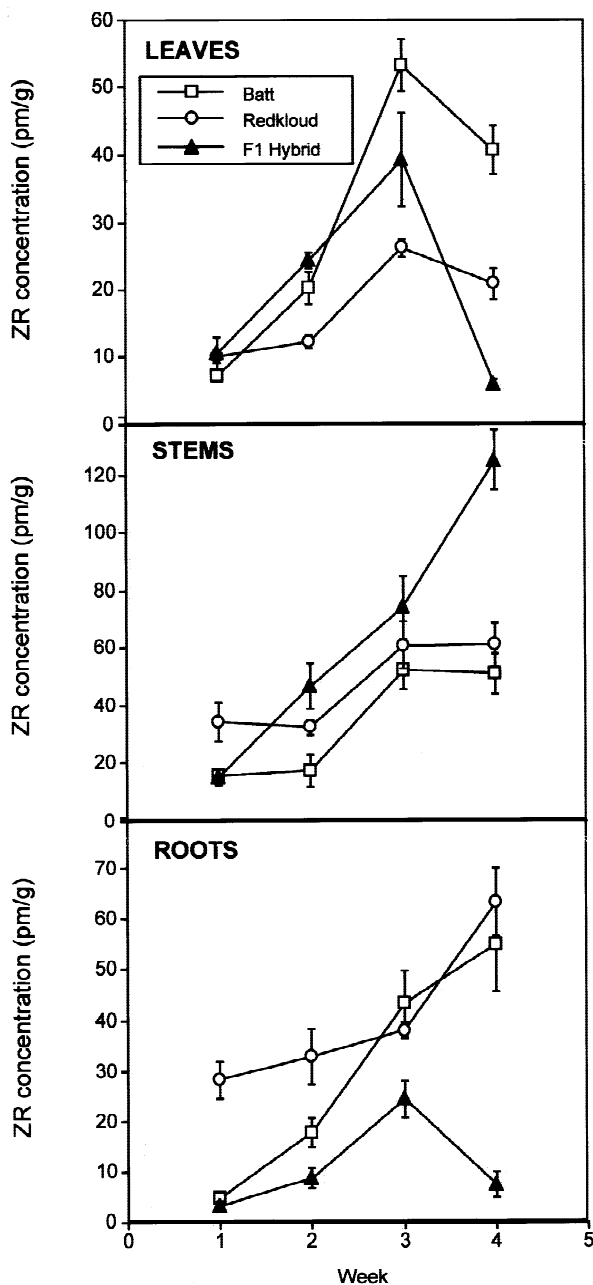

Fig. 5. Changes in ZR-like cytokinin concentrations in roots, stems, and leaves during 4 weeks of growth in the Batt, Redkloud, and F1 hybrids.

used in this study. In addition, evidence indicates that cytokinins are involved in differentiation of vascular tissues during adventitious root development (Aloni 1993). Because significantly higher cytokinin concentrations in F1 hybrid stems were not accompanied by increases in stem mass, it is also possible that high concentrations of cytokinins may deter instead of stimulate stem growth (Badzian and Rybczynski 1994). It is possible that re-
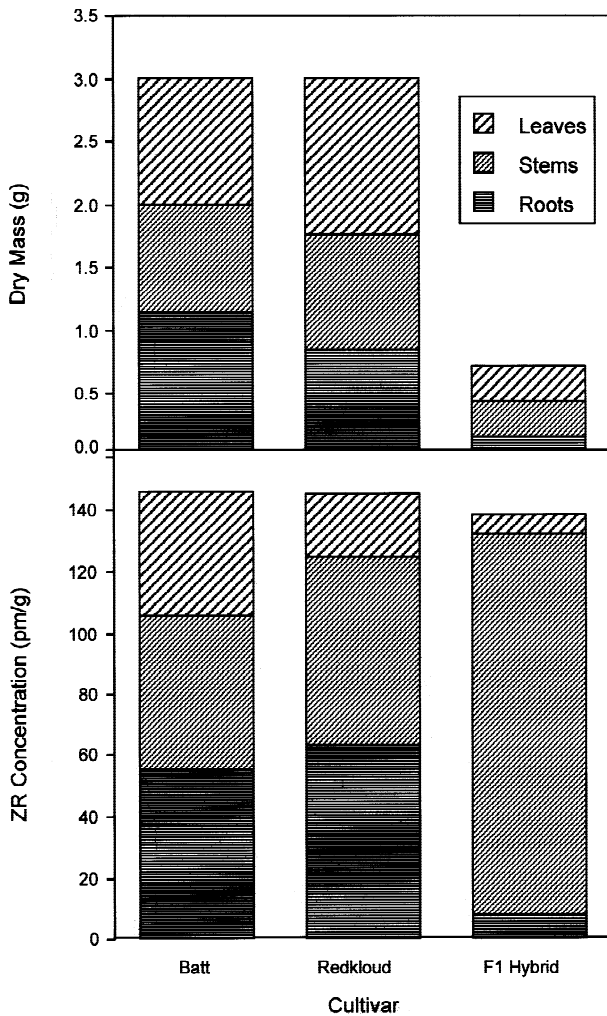
Fig. 6. Dry mass is significantly reduced in leaves, stems and roots in F1 hybrids after 4 weeks of growth compared with Batt and Redkloud; however, ZR-like cytokinin concentrations are significantly increased in stems.

The role of transported cytokinins from roots as an affector of leaf development has received little attention. In this study, significantly lower concentrations of leaf cytokinins were accompanied by severe leaf chlorosis. It is possible, therefore, that leaves of F1 hybrids carrying the DL genes might not receive adequate amounts of cytokinins as the roots deteriorate (Cannell and Jackson 1981, Jackson 1993). In many studies applications of cytokinins affect chlorosis through leaf chlorophyll retention (Banowetz, 1997, Kuang et al. 1992). Kulaeva et al. (1996) and others have discussed a role of endogenous cytokinins as important signals through which roots regulate leaf metabolism and prevent leaf senescence (Garrison et al. 1984, Nooden et al. 1990, Sitton et al. 1967, Soejima et al. 1995). In addition, it has been found that bean leaf expansion can be promoted by cytokinins (Brock and Cleland 1990, Powell and Griffith 1960) although the mechanism is unclear. Cytokinins have also been shown to have a role in cell division. Our understanding of the role of cytokinins is also confounded by the fact that hormone sensitivity is related to tissue competence in the presence of other hormones (Lakshmanan et al. 1997)

Although we do not have a thorough understanding of how incompatibility can develop between closely related cultivars, this study suggests that hormonal relationships may be a factor in the ability to hybridize, thus providing a partial "incompatibility barrier" that may isolate these two bean varieties (Singh and Gutierrez 1984).

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ports from different laboratories differ because of variations in developmental stages of the plants and experimental protocols.

Because normal growth and development depends on interrelationships between cytokinins and other plant growth factors, such as auxin, the role of other hormones cannot be discounted. In addition, the fact that the dose responses for cytokinins are variable is consistent with the idea that plant responses to hormones are governed by more than one factor (Nissen 1988). For example, the presence of adventitious roots may be an indication that stem auxins are increasing (Boerjan et al. 1995, Liu et al. 1993). There are many reports of interactions between cytokinins and auxins, and some of the cytokinins such as zeatin and iso-pentenyladenine have been shown to influence auxin transport (Atzmon et al. 1996).

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