Vertical placement of roots within the soil determines their efficiency of acquisition of heterogeneous belowground resources. This study quantifies the architectural traits of seedling basal roots of bean (*Phaseolus vulgaris*), and shows that the distribution of root tips at different depths results from a combined effect of both basal root growth angle (BRGA) and root length. Based on emergence locations, the basal roots are classified in three zones, upper, middle, and lower, with each zone having distinct architectural traits. The genotypes characterized as shallow on BRGA alone produced basal roots with higher BRGA, greater length, and more vertically distributed roots than deep genotypes, thereby establishing root depth as a robust measure of root architecture. Although endogenous indole-3-acetic acid (IAA) levels were similar in all genotypes, IAA and 1-N-naphthylphthalamic acid treatments showed different root growth responses to auxin because shallow and deep genotypes tended to have optimal and supraoptimal auxin levels, respectively, for root growth in controls. While IAA increased ethylene production, ethylene also increased IAA content. Although differences in acropetal IAA transport to roots of different zones can account for some of the differences in auxin responsiveness among roots of different emergence positions, this study shows that mutually dependent ethylene-auxin interplay regulates BRGA and root growth differently in different genotypes. Root length inhibition by auxin was reversed by an ethylene synthesis inhibitor. However, IAA caused smaller BRGA in deep genotypes, but not in shallow genotypes, which only responded to IAA in the presence of an ethylene inhibitor.

Root architecture (i.e. the three-dimensional configuration of the root system) is an important factor for the acquisition of underground resources (Lynch, 1995). In common bean (*Phaseolus vulgaris*), the root system consists of the primary, basal, lateral, and adventitious roots. The basal roots (BRs), which are specialized secondary roots emerging from the hypocotyl (Zobel, 1996; Basu et al., 2007), together with the primary root constitute a relatively larger portion of the scaffolding for the mature root system (Liao et al., 2001). The length and growth angle of the BRs are two of the most important factors determining root distribution and the consequent acquisition efficiency of belowground resources like nutrients and water, the distribution of which varies with depth (Lynch and Brown, 2001; Ho et al., 2005).

Basal root growth angle (BRGA) and length are regulated by genotype, phosphorus availability, and ethylene (Bonser et al., 1996; Liao et al., 2001; Lynch and Brown, 2001; Basu et al., 2007). BRs in the topsoil are better adapted to low phosphorus availability than BRs in the subsoil, because of higher availability of phosphorus near the soil horizon (Bonser et al., 1996; Liao et al., 2001; Lynch and Brown, 2001; Basu et al., 2007). On the other hand, BR growth into the subsoil is desirable for drought avoidance (Ho et al., 2005).

Phytohormones such as auxin and ethylene play vital roles in modulating root growth and gravitropism, although there are few studies of plagiogravitropic growth responses. Various models have been proposed for ethylene-auxin interplay in root gravi- tropism (Lee et al., 1990; Alonso et al., 2003; Buer et al., 2006) and root growth (Rahman et al., 2001; Swarup et al., 2002; Stepanova et al., 2007). While some studies have reported that ethylene inhibits polar and lateral auxin transport in tissues of shoots (Morgan and Gausman, 1966; Suttle, 1988) and roots (Lee et al., 1990; Prayitno et al., 2006), other studies have reported no effect in hypocotyls and petioles (Abeles, 1966) or a stimulatory effect of ethylene on polar auxin transport in root tissues (Negi et al., 2008, 2010).
According to Stepanova et al. (2005), ethylene triggers auxin synthesis in Arabidopsis (Arabidopsis thaliana) root tip by transcriptional activation of genes coding for both subunits of anthranilate synthase, a phenomenon partially responsible for the inhibition of root growth by ethylene. An indirect assessment of auxin distribution using the auxin-sensitive reporter DR5-GUS by Růžička et al. (2007) also showed that the stimulation of biosynthesis and transport of auxin by ethylene is responsible for inhibition of root elongation. Physiological studies have shown that application of gaseous ethylene inhibits root growth while increasing BRGA in common bean (Basu et al., 2007). Ethylene-auxin interaction also depends on cell type, developmental stage of the organ, and environmental conditions (Stepanova et al., 2007). All of this evidence suggests that auxin and auxin-ethylene interplay could be important for regulating architectural traits of the BRs.

Therefore, the objectives of this study were to (1) quantify the patterns of BRGA, root growth, and root tip depth in different genotypes, (2) analyze the effects of changing levels of auxin on architectural traits of BRs, and (3) describe the role of auxin-ethylene interplay in regulating these traits in common bean during seedling development.

RESULTS

Root Architectural Traits

BRs emerged from a narrow axial region of 0.4 ± 0.1 cm along the lower hypocotyl above the root-shoot interface (Fig. 1). In both deep and shallow genotypes, all of the BRs emerged as protrusions in vertical files (Fig. 1, A and B) within a time window of 4 to 6 h. However, after 2 d of growth, the BRs had very different architectural traits depending on genotype and position of emergence (Fig. 1, C and D). Typically, three axial locations of BR emergence were observed (Fig. 1, A and B). Frequency distribution of the emergence locations of the BRs also showed a trimodal distribution (Fig. 2). Using the nadir values of frequencies between the modes, the emergence locations were clustered in three emergence zones: lower, middle, and upper. Classifications of the BRs in these zones were compared with careful manual classification of the roots based on “whorls” (Basu et al., 2007) by $\kappa$ statistics. For deep and shallow genotypes, $\kappa$ values were 0.97 and 0.87 ($P < 0.001$), respectively.

Measurements of BRGA, root length, and tip depth after 2 d of growth quantified the observed differences in architectural traits of BRs (Fig. 3). As expected, deep genotypes produced roots with significantly smaller
BRGA compared with shallow genotypes from the upper and lower zones, but the middle zones for both genotypes produced BRs of similar BRGA (Fig. 3A). The BRs originating from the higher emergence zones grew with higher BRGA (one-way ANOVA, \( P < 0.001 \)), a result that confirmed similar results from a previous report (Basu et al., 2007). On the other hand, the length of the roots decreased with higher emergence locations (Fig. 3B; one-way ANOVA, \( P < 0.001 \)). The shallow genotypes produced significantly longer BRs than the deep genotypes. Both BRGA and root length contributed to depth distribution of the root tips (Fig. 3C). For straight-growing roots, the relationship among BRGA, root length, and tip depth is expressed as

\[
\text{tip depth} = \text{emergence location} + \text{root length} \times \cos(\text{BRGA})
\]

For curved roots, in Equation 1 root length is replaced with the straight-line distance from the root base to the tip (Fig. 1D). With higher emergence locations, \( \cos(\text{BRGA}) \) increased as the BRGA decreased (Fig. 3A). Simultaneously, the root length increased (Fig. 3B), which resulted in increased tip depth with lower emergence locations (Fig. 3C; one-way ANOVA, \( P < 0.001 \)). For the lower roots of the shallow genotypes, larger BRGA led to smaller \( \cos(\text{BRGA}) \) compared with the deep genotypes (Fig. 3A). However, roots of the shallow genotypes grew longer than the deep genotypes (Fig. 3B). As a result, the tips of the lower roots from the shallow genotypes reached 32.5% deeper than those from the deep genotypes (Fig. 3C). On the other hand, both genotypes produced BRs of similar tip depth from the upper and middle emergence zones.

Endogenous Free Indole-3-Acetic Acid Content and Ethylene Production

To determine if endogenous indole-3-acetic acid (IAA) and ethylene in the BR tissue act as regulators of BRGA and root growth, we measured endogenous IAA as well as ethylene production from the BRs emerging from the upper, middle, and lower zones (Table I). Analysis of IAA content per BR as well as per gram fresh weight of tissue showed that endogenous IAA content did not differ between shallow and deep genotypes or among roots of different emergence locations (Supplemental Table S1). No significant genotypic effect was observed on endogenous ethylene production either. However, endogenous ethylene production was slightly lower \( (P = 0.04) \) in the lower roots compared with the upper ones.

Since wounding (excision) can potentially cause excess ethylene production and hence bias the results, we also compared ethylene evolution from the intact tissues and tissues divided into upper, middle, and lower zones of the emergence region containing the BRs. We observed no significant effect of wounding (excision) on ethylene production, as dividing the root tissues into separate emergence zones did not significantly affect ethylene evolution compared with that of the intact tissue (data not shown).

Responsiveness of BR Architectural Traits to Auxin

The absence of a difference in endogenous IAA among the genotypes with different architectural traits indicates that if auxin has to play any role in regulating these traits, there must be differences in auxin responsiveness among the roots and the genotypes. Therefore, the seedlings were treated with IAA \((0–30 \text{ nmol})\) and 1-N-naphthylphthalamic acid (NPA; \(0–20 \text{ nmol}\)) to examine the responsiveness of root architectural traits to exogenous auxin and an inhibitor of auxin transport, respectively (Fig. 4). Both genotypes showed smaller BRGA at lower emergence zones and vice versa (Fig. 4, A and B; two-way ANOVA, \( P < 0.001 \)), similar to controls (Fig. 3A). In the deep genotypes, hormone treatment also significantly affected BRGA, but not in shallow genotypes (two-way ANOVA, \( P < 0.001 \) in deep, \( P = 0.156 \) in shallow). There was no significant interaction between the main effects, emergence zone, and hormone treatment in either genotype. The BRs in the deep genotypes had significantly smaller BRGA following both IAA and NPA treatments compared with the controls (Fig. 4A; Dunnett’s two-sided test, \( P < 0.001 \)). In the shallow genotypes, however, there was no effect of application of IAA or NPA on BRGA (Fig. 4B).
Two-way ANOVA indicated that both emergence zone and treatments affected root length in both the deep and shallow genotypes ($P < 0.001$). The upper BRs in both deep and shallow genotypes were shorter than the lower BRs (Fig. 4C and D), similar to controls (Fig. 3B). However, the responses of root length to NPA and IAA treatments were different in the deep and shallow genotypes. Controls in the shallow genotypes produced the longest BRs, as treatment with either IAA or NPA reduced root length significantly (Dunnett’s two-sided test, $P < 0.001$; Fig. 4D). In the deep genotypes, while treatment with higher IAA doses inhibited root growth (Fig. 4C; $P < 0.001$ for 20 and 30 nmol), treatment with NPA marginally promoted root growth in the lower and middle roots and slightly inhibited root growth in the upper roots (Fig. 4C).

Variations in BRGA and root length are reflected in tip depth of the IAA- and NPA-treated plants. With the application of IAA, as BRGA reduced in the deep genotypes (Fig. 4A), cos(BRGA) in Equation 1 increased. But at the same time, root length also reduced with IAA (Fig. 4F). As a result, tip depth varied little in deep genotypes following IAA treatment (Fig. 4C). But since BRGA is not significantly affected by IAA treatment in the shallow genotypes, the effect of reduced root length due to IAA treatment (Fig. 4D) is directly reflected in the reduction of tip depth (Fig. 4F; $P < 0.05$ for 20 and 30 nmol). On the other hand, NPA treatment increased tip depth in the deep genotypes (Fig. 4E; $P < 0.05$), a result of lower BRGA due to NPA (Fig. 4A). But NPA reduced tip depth (Fig. 4F; $P = 0.09$ for 10 nmol and $P = 0.03$ for 20 nmol) in the shallow genotypes, a reflection of the inhibition of root growth under NPA.

**Auxin Transport**

The results of IAA and NPA treatment experiments indicated that differences in responsiveness of the BRs to exogenous IAA depended on emergence location. Since exogenous IAA was applied closer to the upper roots, this could be partly explained by differences in auxin transport to roots from different emergence locations. Auxin transport was analyzed using radioactive auxin, [5-$^3$H]IAA, applied in the plastic ring at the hypocotyl, similar to application of IAA or NPA (Fig. 1). There was no difference in radiolabel detection between deep and shallow genotypes. But, as expected, because of the proximity of the upper emergence zone to the site of application (from lower zone, 0.74 ± 0.22 cm; from upper zone, 0.45 ± 0.26 cm), more radiolabel was found in the upper roots than the lower roots (upper, 10,789 ± 967 cpm; lower, 4,978 ± 370 cpm; $P < 0.001$, pooled for both genotypes). This nearly doubling of radiolabel in the upper roots compared with the lower roots may contribute to some extent to the differences in auxin dose response in root architectural traits. In the shallow genotypes, application of 30 nmol of IAA led to 60% and 33% reductions in lengths of upper and lower roots, respectively, whereas the BRs in the deep genotypes shortened by 26% and 22% for the upper and lower emergence zones, respectively, following application of the same amount of IAA.

To assess the effect of ethylene on IAA transport, movement of [3$^3$H]IAA from the hypocotyl to the primary roots and BRs in ethylene-treated seedlings of both genotype classes was measured. Although an
Architectural Traits

Influence of Ethylene Inhibitors on Root Emergence

Ethylene-Auxin Interplay

Auxin could regulate BR architectural traits via alteration of ethylene synthesis or response. To examine if the application of exogenous IAA had any effect on ethylene evolution, endogenous ethylene production rates were measured from the deep and shallow genotypes. Ethylene production from the BRs was significantly higher with external IAA treatment (Table II). The effect of ethylene on free IAA content was quantified by gas chromatography-mass spectrometry (Engelberth et al., 2003; Schmelz et al., 2003). There was an approximately 30% increase (P < 0.02) in IAA content per BR following ethylene treatment.

Ethylene-Auxin Interplay

To examine the changes in architectural traits of the BRs, the seedlings were treated with both IAA and the ethylene synthesis inhibitor aminoethoxyvinylglycine (AVG). A preliminary experiment showed that 1.2 nmol (60 μM) of AVG inhibited ethylene production from the BRs by 80%. Therefore, BRGA, root length, and tip depth were measured after treatment with 1.2 nmol of AVG + 30 nmol of IAA (Fig. 5). In the deep genotypes, application of 30 nmol of IAA reduced BRGA, but application of AVG + IAA had no additional effects (Fig. 5A). In shallow genotypes, opposite effects were observed: 30 nmol of IAA alone had very little effect on BRGA, but addition of AVG + IAA reduced BRGA (Fig. 5B). AVG completely reversed the IAA-induced effect on root length in deep genotypes when compared with the control (Fig. 5C). However, in shallow genotypes, AVG + IAA partially reversed the shortening of roots due to IAA alone (Fig. 5D). Whereas IAA alone reduced tip depths of lower roots, addition of AVG did not alter IAA effects on tip depths of either deep or shallow genotypes (Fig. 5, E and F).

Comparison of endogenous IAA content (per g fresh weight of tissue and per BR) and endogenous ethylene production (per g fresh weight of tissue) following ethylene treatment.

Table I. Comparison of endogenous IAA content (per g fresh weight of tissue and per BR) and endogenous ethylene production (per g fresh weight of tissue)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Emergence Zone</th>
<th>Endogenous IAA Measure</th>
<th>Endogenous Ethylene Production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ng g⁻¹ fresh wt</td>
<td>ng BR⁻¹</td>
</tr>
<tr>
<td>Deep</td>
<td>Upper</td>
<td>26.16 ± 6.0</td>
<td>0.23 ± 0.066</td>
</tr>
<tr>
<td>Deep</td>
<td>Middle</td>
<td>28.90 ± 0.9</td>
<td>0.28 ± 0.006</td>
</tr>
<tr>
<td>Deep</td>
<td>Lower</td>
<td>28.04 ± 0.5</td>
<td>0.28 ± 0.024</td>
</tr>
<tr>
<td>Shallow</td>
<td>Upper</td>
<td>25.78 ± 3.1</td>
<td>0.21 ± 0.006</td>
</tr>
<tr>
<td>Shallow</td>
<td>Middle</td>
<td>28.61 ± 4.2</td>
<td>0.27 ± 0.062</td>
</tr>
<tr>
<td>Shallow</td>
<td>Lower</td>
<td>27.91 ± 5.4</td>
<td>0.28 ± 0.003</td>
</tr>
</tbody>
</table>

DISCUSSION

This study presents detailed quantitative assessment of architectural traits (e.g. growth angle, root length, and tip depth) of BRs of common bean and the effects of auxin-ethylene cross talk on these traits. These architectural traits play key roles in conformation of the root system and the consequent efficiency of acquisition of belowground resources. BRs emerge on day 3 after imbibition when the primary root is about 2 to 3 cm long. When unimpeded, the BRs exhibit plagiogravitropic growth and tend to maintain their growth trajectory for the next 24 h; eventually, they reorient their direction of growth by exhibiting higher or lower BRGA (Bonser et al., 1996; Basu et al., 2007).

Quantitative Measure of BR Emergence Zones

BRs have been observed to emerge from two to three distinct whorls along the lower hypocotyl (Basu et al., 2007), but the emergence zones are not perfectly aligned (e.g. roots l₁, l₂, and l₃ in Figure 1A emerge from the lower whorl, but their emergence occurs at different positions along the hypocotyl). Quantitative assessment of the emergence location revealed three emergence zones that matched very well with the manual classification. These zones allow objective classification of the roots that might be difficult and ambiguous to classify by visual examination. For example, in Figure 1, C and D, it is difficult to objectively identify the originating zone of each BR by visual observation alone, especially when the roots are more than 1 cm long, although in the emerging seedlings they are identifiable (Fig. 1, A and B). But the
quantitative demarcation of each emergence zone makes it easy and objective to identify the zone of emergence of each BR. Because of distinct BRGA for each zone, the roots tend to occupy specific soil depths during growth. Consequently, for the nutrients that are nonuniformly distributed in the soil, one can anticipate specific roles of BRs emerging from the specific zones. These zones can be used to characterize the physiology and function of each group of BRs.

**Characterization of Root Systems by Depth**

The previous classification of genotypes as shallow or deep was based on BRGA alone (Bonser et al., 1996; Liao et al., 2001). Calculation of tip depth provides a more direct estimation of the vertical root position and hence allows better categorization of the root system. As explained using Equation 1, the depth of the BRs is affected by both BRGA and root length. As a result, the lower roots from the shallow genotypes actually had deeper tips than those of the deep genotypes, but roots from the upper zone had similar tip depths in both genotype classes. In addition, root length and angle also determine the horizontal placement of the BRs, with greater length and larger BRGAs spreading the roots away from the primary root and vice versa. Therefore, these results indicate that compared with the deep genotypes, the shallow genotypes have a more “spread out” root system, vertically and horizontally, which not only helps the shallow genotypes adapt better to vertically heterogeneous distribution of soil resources but also reduces intraplant competition (Lynch and Brown, 2008).

**Auxin-Mediated Changes in Root Architectural Traits**

The presence of a variety of root growth angles in various bean genotypes (Bonser et al., 1996; Liao et al., 2001; Basu et al., 2007) and the known role of auxin in regulating gravitropic responses (Luschnig et al., 1998; Marchant et al., 1999; Friml et al., 2002) prompted us to examine whether endogenous IAA in the BRs could be responsible for differences in their architectural traits. However neither endogenous IAA concentration (ng g⁻¹ fresh weight) nor content (ng BR⁻¹) was found to be different between the shallow and deep genotypes, indicating that total endogenous IAA is insufficient to account for the variation in BRGA or growth rates. Therefore, auxin responsiveness is likely to regulate the architectural traits of the BRs.

Our experiments with IAA and NPA treatments showed that auxin responsiveness indeed varied with genotypes as well as specific architectural traits. Shallow genotypes appeared to have optimal auxin for root growth, since growth was reduced by either IAA or NPA treatment, but not so in deep genotypes. Instead, auxin content in the roots emerging from the middle and lower zones of deep genotypes appeared to be supraoptimal for growth, since treatment with NPA slightly increased root length, while treatment with IAA reduced it significantly. The roots from the upper emergence zone in deep genotypes showed similar responsiveness to that of shallow genotypes, although the effects were not significant. Acropetal auxin transport has been shown to influence root growth in Arabidopsis (Reed et al., 1998), while basipetal transport controls root gravitropism (Rashotte et al., 2000). We applied IAA and NPA at the hypocotyl above the basal rooting zone. Therefore, it appears that exogenous IAA and NPA at the hypocotyl above the basal rooting zone. Therefore, it appears that exogenous IAA and NPA had stronger influence on acropetal than basipetal transport of auxin, leading to greater auxin responsiveness of root length than BRGA in shallow genotypes. Comparatively, deep genotypes appeared to have optimal auxin concentration for BRGA, as BRGA was reduced by either IAA or...
NPA (Fig. 4A). However, BRGA in shallow genotypes was relatively insensitive to IAA or NPA treatment, indicating that basipetal auxin transport may have been affected by IAA or NPA treatment in deep genotypes but not in shallow genotypes.

Transport of auxin studied with [5-3H]IAA showed that the proximity of the application point leads to nearly double auxin content in the roots of the upper zone relative to the lower zone, but there is no difference in auxin transport between deep and shallow genotypes. Therefore, transport of exogenous auxin may account for the differences in response to application of IAA (and possibly NPA as well) between roots of different zones, but it does not explain the variation in responses between deep and shallow genotypes; rather, it points to differences in responsiveness of the genotypes to auxin.

**Ethylene-Auxin Cross Talk**

Ethylene has been repeatedly invoked as an important modulator of gravity responses (Chadwick and Burg, 1967; Wheeler and Salisbury, 1981; Lee et al., 1990; Madlung et al., 1999) in addition to auxin. Here, we show that IAA stimulated ethylene production (Table II), as expected from earlier observations (Abeles et al., 1992; Abel et al., 1995; Woeste et al., 1999). Similarly, application of gaseous ethylene also increased endogenous IAA content, a result consistent with earlier reports showing that ethylene application enhanced IAA synthesis (Stepanova et al., 2007; Swarup et al., 2007) as well as IAA transport (Negi et al., 2008). It has also been shown that, similar to IAA, gaseous ethylene inhibited root growth of the same genotypes (Basu et al., 2007). In addition, ethylene had a strong effect on BRGA as well (Basu et al., 2007). Here, we show that IAA reduces BRGA, but only in the deep genotypes. Ethylene synthesis inhibition with AVG neither reversed nor enhanced the auxin effect on BRGA in the deep genotypes. Therefore, it seems that the effect of AVG was insufficient to alter the auxin effect on BRGA in deep genotypes. However, AVG reduced BRGA of shallow genotypes, indicating that BRGAs of shallow genotypes are more sensitive to ethylene, confirming previous observations (Basu et al., 2007).

Comparison of root lengths following treatments with IAA and IAA + AVG with controls showed similar effects in both deep and shallow genotypes. The inhibition by IAA was reversed completely in deep genotypes and partially in shallow genotypes due to the inhibition of ethylene biosynthesis by AVG treatment along with IAA. It has been reported that auxin signaling is downstream of the ethylene signal transduction pathway (Roman et al., 1995; Stepanova

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Deep Genotype (RIL7)</th>
<th>Shallow Genotype (RIL57)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upper</td>
<td>Middle</td>
</tr>
<tr>
<td>Control</td>
<td>46.71 ± 3.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.30 ± 2.2</td>
</tr>
<tr>
<td>IAA</td>
<td>63.01 ± 4.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.68 ± 3.6</td>
</tr>
</tbody>
</table>

Table II. Effect of exogenous IAA (30 nmol) on ethylene production by tissue segments of emergence zone-bearing young BRs

Values shown are means ± se of four to seven plants for each group. Differences in ethylene production from control and IAA-treated roots for each emergence zone and each genotype as determined by t test are indicated as follows: <sup>a</sup> P < 0.05, <sup>b</sup> P < 0.01.
et al., 2005), suggesting that ethylene regulates root growth via auxin. These observations, therefore, led to the following hypothetical model explaining ethylene-auxin interplay in regulating root growth.

A Hypothetical Model for Ethylene-Auxin Cross Talk in Regulating BR Architecture

Based on the results from our experiments together with the observations reported in the literature, we propose a hypothetical model to explain ethylene-auxin interplay in regulating BR growth in common bean (Fig. 6). Since the shallow genotypes have optimal auxin for root growth, addition of IAA or NPA makes auxin content supraoptimal or suboptimal, respectively, inhibiting root growth. However, the deep genotypes naturally have supraoptimal auxin for root growth. Exogenous IAA inhibits root growth, but NPA may reduce auxin content to optimal or suboptimal levels, depending on the dose of NPA. Since NPA was applied at the hypocotyl, the NPA effect may have been stronger in the upper BRs than in the middle and lower BRs. As a result, auxin content is likely driven to suboptimal levels, slightly inhibiting root growth in the upper roots of the deep genotypes. In the middle and lower roots of the deep genotypes, NPA tends to reduce auxin transport toward optimal levels, marginally promoting root growth. Therefore, root growth response to auxin is dependent on the endogenous concentration of auxin relative to the auxin-versus-root growth curve (i.e. whether auxin concentration is optimal, suboptimal, or supraoptimal for root growth in controls).

Auxin stimulates ethylene synthesis (Abeles et al., 1992; Abel et al., 1995; Woeste et al., 1999; this work), while ethylene promotes auxin synthesis (Stepanova et al., 2007; Swarup et al., 2007; this work) and transport (Negi et al., 2008, 2010). Ethylene is also known to have stimulatory effects on both acropetal and basipetal auxin transport (Buer et al., 2006; Ružička et al., 2007; Negi et al., 2008), regulating root growth and gravitropism, respectively. Our experiments with [5-3H]IAA indicated that application of IAA at the hypocotyl above the basal rooting zone directly influenced acropetal auxin transport, which affected root growth. In addition, IAA treatment also increased ethylene production, which in turn can affect basipetal transport of auxin and thereby potentially influence the graviresponse of the BRs. Therefore, this mutually dependent ethylene-auxin interaction may be the key mechanism of variations in BRGA due to exogenous IAA and IAA + AVG. However, the difference in response of BRGA to IAA and IAA + AVG between deep and shallow genotypes indicates a more complex genotype-dependent interaction between auxin and ethylene in regulating graviresponse of the BRs, which this study does not completely resolve.

Although our study was designed initially to test the effect of different phosphorus treatments on BR architectural traits, in both this and the previous work (Basu et al., 2007), there were no significant effects of phosphorus on root architecture. Previous work with older seedlings showed that genotypes vary in their response to phosphorus (Bonser et al., 1996; Liao et al., 2001). Therefore, a more detailed study with older plants or different genotypes is necessary to explore BR architectural plasticity in response to nutrient availability. Furthermore, although the BRs from all zones tend to emerge within a time span of 4 to 6 h, the small temporal difference in emergence time of each root may contribute to the differences in root length after 48 h. As the development of the BRs is a continuous process, it is very difficult to pinpoint the exact timing of emergence of the BRs. Therefore, this study does not quantify the timing of the emergence of BRs.
and a detailed kinematic study is planned to establish the effect of temporal difference in variations in root growth on root length and consequent root architecture. Since the study focuses on early seedlings alone in the two-dimensional growth pouch, further investigations are necessary to explore how these early developmental features of BRs translate to mature root systems in the native three-dimensional environment.

In conclusion, this study provides a quantitative description of architectural traits of BRs of common bean seedlings and contrasts these between two genotype classes. The hormonal cross talk regulating the architectural traits of roots is complex. This study explores this complexity of ethylene-auxin interaction in regulating root growth and builds a framework for future molecular studies.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Six genotypes (parents B98311 and TLP19 and recombinant inbred lines [RILs] 15, 57, 7, and 76) of bean (Phaseolus vulgaris) were selected from the L88 population developed by Dr. Jim Kelly at Michigan State University (Frahm et al., 2004). B98311 and RIL7 and RIL76 have deep root systems (BRGA of 41.7±14%), and TLP19 and RIL15 and RIL57 have shallow root systems (BRGA of 56.4±18%; Basu et al., 2007).

Seeds were surface sterilized with 6% sodium hypochlorite, rinsed with distilled water, and scarified. Seeds were placed in darkness at 28°C in a germination chamber for 2 d in rolled germination paper (25.5×73.5 cm) and compared with [2H5]IAA as internal standard. Methanol chemical ionization (Trace GC 2000 attached to a GCQ mass spectrometer; Thermo Finningan) and Student’s t test were used to detect significant differences in architectural traits of BRs between deep and shallow genotypes, whereas one-way ANOVA was used to identify differences between roots of different zones. Two-way ANOVA followed by Dunnett’s two-tailed t test were used to detect significant differences between control and treatments associated with genotypes and emergence zones. Effects of genotype, emergence location, and statistical analysis.

Measurement of Ethylene Production

Endogenous ethylene production was measured from the tissue segments of different emergence zones bearing BRs of the control and auxin-treated (30 nmol) seedlings at 48 h. The tissues were excised and immediately enclosed in 9-mL air-tight vials at 25°C. A 1-mL volume of the head space was taken from the vials 2 h later and then injected into a gas chromatograph (HP6890; Hewlett-Packard). To assess the effect of wounding (excision) on ethylene production, ethylene was measured from intact tissue (whole segment of the basal rooting zone) with BRs. These results were compared with the ethylene evolution from the wounded tissues that had been cut into three separate zones of emergence containing BRs and analyzed together.

Treatment with Ethylene Inhibitors

Seedlings were exposed to an inhibitor of ethylene biosynthesis, AVG, together with IAA. Concentrations of 60 μM (1.2 nmol) AVG and 30 nmol of IAA were added in the ring at the hypocotyl (Fig. 1) at 0 and 24 h.

Auxin Transport Analysis

Auxin transport was assessed using radioactive auxin [5-3H]IAA (25 Ci mmol−1; American Radiolabeled Chemicals). Twenty microliters of the stock solution (by diluting 40 μM [5-3H]IAA with 1.5 ml cold IAA, which is equivalent to 30 nmol, to make a total volume of 3 mL) was placed in the plastic ring at the hypocotyl (Fig. 1). Seedling segments were harvested to evaluate the transport of labeled IAA to the BR segments. Tissue samples were transferred to separate vials containing 10 mL of Biosafe II, a biodegradable and nonflammable scintillation fluid. Counts of radioactivity were measured for 2 min using a scintillation counter (1500 Tricarb; Packard). To examine the effect of ethylene on IAA transport, the radioactive seedlings in the pouch were treated with ethylene inside an air-tight plexiglass chamber after application of [3H]IAA before harvesting to determine radioactivity.

Data Analysis

The BRs emerged from up to three zones along the hypocotyl (Fig. 1, C and D) previously referred to as whorls (Basu et al., 2007). These zones were identified quantitatively from frequency distributions of emergence locations measured relative to the lowest emerging BR. To compare how the emergence zones match with the whorls, an experienced researcher manually identified the whorls of emergence of the BRs from closeup views. Each experiment consisted of two to six contrasting genotypes in two classes (shallow and deep). Although two contrasting nutrient solutions containing low and high phosphorus were used, there was no statistically significant effect of phosphorus treatment on root architectural traits. Therefore, in this entire study, data were pooled over both high- and low-phosphorus treatments.

Statistical Analysis

The x statistic was used as a measure of concordance between quantitative classification of BRs in emergence zones and manual identification of contrasting whorls. Student’s t test was used to detect significant differences in architectural traits of BRs between deep and shallow genotypes, whereas one-way ANOVA was used to identify differences between roots of different zones. Two-way ANOVA followed by Dunnett’s two-tailed t test were used to detect significant differences between control and treatments associated with genotypes and emergence zones. Effects of genotype, emergence location, and statistical analysis.
dose of hormone and inhibitors on growth angle, root length, and tip depth were tested at the 95% confidence level. Statistical analysis for this study was carried out with SPSS 13.0 (SPSS).

Supplemental Data
The following materials are available in the online version of this article.

Supplemental Table S1. Two-way ANOVA for endogenous IAA content and ethylene production from BRs of deep and shallow genotypes.

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LITERATURE CITED


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