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Root-localized phytochrome chromophore synthesis is required for photoregulation of root elongation and impacts root sensitivity to jasmonic acid in *Arabidopsis thaliana*

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ABSTRACT

Plants exhibit organ- and tissue-specific light responses. To explore the molecular basis of spatial-specific phytochrome-regulated responses, a transgenic approach for regulating synthesis and accumulation of the phytochrome chromophore phytochromobilin (PΦB) was employed. In prior experiments, transgenic expression of the biliverdin reductase (BVR) gene was used to metabolically inactivate biliverdin IXα, a key precursor in the biosynthesis of PΦB, and thereby to render cells accumulating BVR phytochrome deficient. Here, we report analyses of transgenic Arabidopsis thaliana lines with distinct patterns of BVR accumulation dependent upon constitutive or tissue-specific, promoter-driven BVR expression that have resulted in insight on a correlation between root-localized BVR accumulation and photoregulation of root elongation. Plants with BVR accumulation in roots and a PΦB-deficient hy2-1 mutant exhibit roots that are longer than those of wild-type plants under white illumination. Additional analyses of a line with root-specific BVR accumulation generated using a GAL4-dependent bipartite enhancer-trap system confirmed that PΦB or phytochromes localized in roots directly impact light-dependent root elongation under white, blue and red illumination. Additionally, roots of plants with constitutive plastid-localized or root-specific cytosolic BVR accumulation, as well as phytochrome chromophore-deficient hyl-1 and hy2-1 mutants exhibit reduced sensitivity to the plant hormone jasmonic acid (JA) in JA-dependent root inhibition assays, similar to the response observed for JA insensitive mutants jar1 and myc2. Our analyses of lines with root-localized phytochrome deficiency or root-specific phytochrome depletion have provided novel insight(s) into the roles of root-specific PΦB, or phytochromes themselves, in the photoregulation of root development and root sensitivity to JA.
INTRODUCTION

Light can have opposing effects in different plant tissues: light exposure inhibits hypocotyl elongation, whereas light promotes cotyledon expansion and root development (reviewed by Bou-Torrent et al., 2008). The occurrence of distinct organ- or tissue-specific, light-regulated processes in plants is supported by tissue-specific microarray analyses. For example, distinct subsets of light-regulated genes can be identified from cotyledons vs. roots in Arabidopsis (Jiao et al., 2005; Ma et al., 2005) or from shoots vs. roots in rice (Jiao et al., 2005). Red (R) and far-red (FR) light-absorbing phytochromes are expressed in roots of Arabidopsis (Tóth et al., 2001; Salisbury et al., 2007), as are blue (B) light photoreceptors (Tóth et al., 2001; Galen et al., 2007). UV-B is also absorbed by roots and its absorption impacts root and seedling development in Arabidopsis (Tong et al., 2008; Leasure et al., 2009). The light-dependent growth responses of roots and localization of photoreceptors in roots themselves implicate root-localized light perception as an important attribute for photomorphogenic adaptation in plants.

Light can penetrate several millimeters into the upper layers of soil, and the depth of light penetration depends on the soil composition (Tester and Morris, 1987; Mandoli et al., 1990). These findings suggest that roots can perceive light directly in natural environments and that root development is likely impacted by light under certain physiological conditions. Indeed, light is expected to function as a useful signal for roots to perceive their depth in the soil stratum. Certainly, phytochromes in seeds perceive light that penetrates the soil as it is well recognized that seed germination is impacted by light (reviewed by Shinomura, 1997; Seo et al., 2009). In addition to localized detection of light, roots may also be impacted by light perceived by aerial plant tissues through internal tissue piping effects (Mandoli and Briggs, 1982).

Recent results indeed have demonstrated that photoreceptors, including phytochromes and B-light absorbing phototropins, affect root growth and development in natural environments. Galen et al. (2007) have shown that phototropin localization in roots is correlated with increased root growth efficiency in the natural environment. In soil-grown plants, phytochrome also has been shown to have a role in root development;
i.e., phyB regulates the initiation of lateral roots for plants grown on a soil/compost mixture (Salisbury et al., 2007). This finding suggests that roots in a more natural environment, i.e., growing under soil, exhibit phytochrome-dependent regulation of root development.

In addition to a role in root development, the photoreceptor phytochrome also regulates other distinct aspects of photomorphogenesis in plants, including seed germination, cotyledon expansion and leaf development, inhibition of hypocotyl elongation, and the induction of flowering (Chen et al., 2004; Mathews, 2006). In higher plants, photoactive phytochrome consists of an apoprotein covalently attached to the linear tetrapyrrrole chromophore, phytochromobilin (PΦB; Terry et al., 1993). All higher plant phytochromes, including phyA to phyE in Arabidopsis thaliana (Sharrock and Quail, 1989; Clack et al., 1994), use this chromophore for their photoregulatory activities (Terry et al., 1993).

Apart from the abovementioned role for phyB, the roles of phytochromes in roots have not been studied extensively in soil-grown plants or natural environments. However, roles for phytochromes in the regulation of distinct aspects of root development have been identified definitively in controlled photobiological studies. For example, R light absorbed by root-localized phyA and phyB impacts root phototropism (Kiss et al., 2003) and inhibits root elongation rates (Correll and Kiss, 2005) in Arabidopsis. Recent microarray analyses for roots exposed to R light indicate that the expression of many genes, including a number of genes involved in photomorphogenesis and root development, are regulated by light in roots (Molas et al., 2006). FR light also impacts root growth, i.e., stimulating phyA-dependent root elongation in Arabidopsis seedlings (Kurata and Yamamoto, 1997).

Results regarding the impact of global phytochrome deficiency on root elongation in chromophore biosynthetic mutants, which exhibit deficiencies in all members of the phytochrome family, are unresolved. Previously, a hy1 (hy1[21.84N]) mutant was shown to have longer roots than the WT control under white (W) light illumination (Muramoto et al., 1999), whereas in separate reports additional hy1 mutants (hy1-100 and hy1-101) appear to have slightly shorter roots than the Col-0 WT parent (Zhai et al., 2007). Notably, HY1, which encodes a heme oxygenase, is expressed in roots (Davis et al.,
1999; Davis et al., 2001; Emborg et al., 2006) and expression of HY1 is down-regulated by treatment with the plant hormone jasmonic acid (JA; Zhai et al., 2007). JA is a regulator of plant growth and development, in addition to its recognized role in regulating defense responses (Browse, 2005). JA generally inhibits leaf (Zhang and Turner, 2008) and root growth (Staswick et al., 1992) in Arabidopsis. Inhibition of root growth by JA has been widely used to identify mutants impaired in JA-biosynthesis and/or signaling based on insensitivity to root growth inhibition upon exogenous application of JA or JA analogs. Notably, phytochrome chromophore deficiency is associated with altered JA-mediated root inhibition (Zhai et al., 2007), suggesting a link between phytochrome chromophore biosynthesis and JA signaling pathways.

An initial report on a molecular link between JA and phytochrome signaling described a mutant displaying an FR-specific long hypocotyl phenotype, which was found to have a mutation in the FAR-RED-INSSENSITIVE219 (FIN219) gene (Hsieh et al., 2000). The fin219 mutation was found to be allelic to the jar1 mutation (Staswick et al., 2002). JAR1 encodes a JA-amino synthetase, catalyzing the conjugation of JA to Ile, required for optimal signaling in jasmonate responses in Arabidopsis (Staswick and Tiryaki, 2004). Both jar1 and fin219 mutants display a long hypocotyl phenotype under continuous FR (FRc) compared to WT (Chen et al., 2007), suggesting that mutants with defects in JA biosynthesis also are impaired in phytochrome-mediated signaling in FR. Additionally, the PHYTOCHROME AND FLOWERING TIME1 (PFT1) gene, which encodes a component that negatively regulates phytochrome signaling, is required for JA-dependent expression of defense genes in Arabidopsis (Kidd et al., 2009). It appears that phyA is a critical player in the interaction with JA as a phyA mutant recently has been shown to be hyposensitive in JA root inhibition assays (Robson et al., 2010).

Here, we describe the characterization of light-dependent root development in transgenic Arabidopsis plants exhibiting induced phytochrome chromophore deficiency. Previous studies using tissue-specific promoters to induce localized phytochrome chromophore deficiencies through targeted expression of the biliverdin reductase gene (BVR) enabled the identification of distinct light-impaired phenotypes (Montgomery, 2009; Warnasooriya and Montgomery, 2009; Warnasooriya et al., 2011). To examine the role of root-localized phytochromes in the photoregulation of root growth and
development in Arabidopsis, we analyzed roots of promoter-driven BVR lines in addition to a newly generated GAL4-based enhancer-trap transactivation BVR line exhibiting root-specific phytochrome chromophore deficiency. The analyses of these plants, which all exhibit differential accumulation of a phytochrome chromophore-degrading enzyme in distinct tissues, provide insight into the impact of shoot- versus root-derived phytochrome signals on photoregulation of root lengths and indicate that root-localized phytochrome chromophore deficiencies are correlated with light-dependent defects in root development and root sensitivity to JA in A. thaliana.

RESULTS

Phytochrome or phytochromobilin impacts root elongation in Arabidopsis

We compared light-dependent root lengths in lines exhibiting phytochrome chromophore deficiencies, using either constitutive (Montgomery et al., 1999) or tissue-specific, promoter-driven (Warnasooriya and Montgomery, 2009) BVR lines. We confirmed the accumulation of BVR protein in the roots of these lines using anti-BVR immunohistochemical analysis (Fig. 1). We detected BVR accumulation in the roots of only the 35S::pBVR3 and 35S::cBVR1 lines (Fig. 1B and 1C), as previously determined using immunoblot analyses (Warnasooriya and Montgomery, 2009). We determined that only those lines that have BVR-induced phytochrome chromophore deficiencies in roots themselves, i.e., 35S::pBVR3 or 35S::cBVR1, had noticeably longer roots relative to No-0 WT when grown under continuous white (Wc) illumination (Fig. 2A). When we quantified root lengths for No-0 WT and BVR transgenic lines, we determined that 35S::pBVR3 and 35S::cBVR1 lines have significantly longer roots (p<0.0001) than the No-0 WT parent (Fig. 2B). The roots of 35S::pBVR3 and 35S::cBVR1 seedlings are ~45% and ~90% longer than the roots of No-0 WT seedlings, respectively. Notably, the roots of CAB3::pBVR2 seedlings, which exhibit mesophyll-specific phytochrome deficiency (Warnasooriya and Montgomery, 2009), also were marginally longer by ~21% than those of No-0 WT (p= 0.0165; Fig. 2B), though this difference was not to the same degree as observed for 35S::pBVR3 and 35S::cBVR1. By contrast, the root lengths of
MERI5::pBVR1 seedlings, which exhibit shoot-apex-specific BVR accumulation (Warnasooriya and Montgomery, 2009), were not significantly different from those of No-0 WT (p=0.324, Fig. 2B).

As the differences observed for promoter-driven BVR lines indicated that highly significant differences observed in root lengths were for those lines in which the phytochrome chromophore was depleted in roots themselves, we generated a line exhibiting root-specific phytochrome chromophore deficiency using a GAL4-based bipartite enhancer trap transactivation system that has been successfully employed in Arabidopsis (Laplaze et al., 2005), as well as in rice (Johnson et al., 2005). M0062 exhibits root-specific GFP expression and, thus, GAL4 accumulation (http://www.plantsci.cam.ac.uk/Haseloff/assembly/page167/index.html; Haseloff, 1999). After a cross of the M0062 enhancer trap line with a GAL4-responsive UASBVR line, we isolated a transgenic BVR enhancer-trap line, M0062/UASBVR, that exhibits root-localized BVR protein accumulation (Fig. 3G). In this line, which has root-localized \( \Phi B \) deficiency, and thus root-specific holophytochrome deficiencies, we observed that roots of Wc-grown seedlings were longer than the C24 ecotype background (Fig. 2A), similar to the response observed for the 35S promoter-driven BVR lines. When quantified, the roots of M0062/UASBVR seedlings are significantly longer, i.e., 20% longer, than C24 (p=0.0066; Fig. 2B).

In comparative studies, we examined root lengths of two phytochrome chromophore-deficient mutants, \( hy1-1 \) and \( hy2-1 \) (Fig. 2A). Whereas roots of the \( hy1-1 \) mutant were on average shorter than the C20 WT parent by ~15%, roots of the \( hy2-1 \) mutant were on average ~23% longer than the roots of C20 WT (Fig. 2B). Neither of these observed differences in root length was found to be statistically significant (p=0.1524 and p=0.0859, respectively), as compared to the lengths of roots of the C20 WT parent. Ecotypic differences in roots lengths were observed for WT lines, with Col-0 WT having longer roots than other ecotypes (Supplemental Fig. S1), as previously described (Beemster et al., 2002; Passardi et al., 2007).

**Phytochrome-dependent regulation of root elongation under specific wavelengths of light**
To determine the effect of phytochrome depletion in roots on the elongation of roots under distinct wavelengths of light, we grew seedlings under continuous blue (Bc) red (Rc), and far-red (FRc) illumination and assessed the lengths of roots relative to cognate WT seedlings. We observed roots that were significantly longer in lines with root-localized BVR accumulation, i.e. 35S::pBVR3, 35S::cBVR1 and M0062/UASBVR, relative to cognate WT parents under Bc illumination (Fig. 4A). Significantly longer roots also were observed for a phyA mutant relative to C20 WT (Fig. 4A). A role for phytochromes in the regulation of root length under B light has been previously postulated (Canamero et al., 2006).

Under Rc illumination, the M0062/UASBVR lines exhibited significantly longer roots than its parent C24 WT (Fig. 4B). The phyB mutant had significantly shorter roots than WT under these conditions, a phenotype previously observed in R (Shin et al., 2010). Notably, under FRc, smaller and largely non-significant differences in root lengths were observed for BVR lines (Fig. 4C), suggesting that globally phytochromes localized in roots responded more to R and B illumination. However, 35S::cBVR1 seedlings and phyA seedlings were significantly shorter than WT under FRc (Fig. 4C), suggesting a role for shoot-derived phyA on regulation of root length. The shorter roots observed for a phyA mutant have been previously reported (Kuratu and Yamamota, 1997; Shin et al., 2010).

**Root-specific BVR phytochrome depletion does not impact light-dependent changes in hypocotyl elongation**

The observation that constitutive BVR-expressing seedlings and CAB3::pBVR seedlings exhibit elongated hypocotyls in Rc, FRc and Bc light indicates a lack of phytochromes within the shoots of these seedlings (Lagarias et al., 1997; Montgomery et al., 1999; Montgomery, 2009; Warnasooriya and Montgomery, 2009). To confirm the root-specific inactivation of phytochromes in the M0062/UASBVR line and rule out residual activity of the BVR enzyme in the shoots of these seedlings, we assessed whether light-dependent hypocotyl inhibition was impacted in this line under Bc, Rc, or...
The positive control 35S::cBVR1 line exhibited a significantly longer hypocotyl compared to the No-0 WT parent under Bc, Rc, and FRc (p>0.0001 for all conditions, Fig. 5) as previously reported (Montgomery et al., 1999). By comparison, the hypocotyl lengths of the M0062/UASBVR line were not significantly different from C24 WT under Bc (p=0.7146), Rc (p=1.0) or FRc (p=1.0) (Fig. 5), confirming root-specific impacts of BVR-mediated, phytochrome deficiencies in the M0062/UASBVR line.

**Root-localized ΦB or phytochromes impact Arabidopsis root elongation response to jasmonates**

Previous results indicated that phytochrome chromophore-deficient plants exhibit altered JA accumulation that is correlated with changes in root elongation (Zhai et al., 2007). Inhibition of root elongation is promoted by JA and bioactive derivatives of JA (Staswick et al., 1992). We used our collection of BVR lines that exhibit phytochrome chromophore deficiency in various tissues to determine whether phytochrome chromophore deficiency in the root itself is correlated with the observed link between changes in JA levels and associated root elongation responses. Specifically, we conducted experiments to investigate the impact of root-localized phytochrome chromophore depletion on the elongation response of roots in JA-dependent root inhibition assays. We grew No-0 WT, 35S::pBVR3, 35S::cBVR1, CAB3::pBVR2 and MERI5::pBVR1 seedlings in the presence and absence of 20 µM MeJA and measured the lengths of roots. In our analyses, plants with root-localized phytochrome chromophore deficiencies were hyposensitive to JA (Fig. 6A). Seedlings of the lines 35S::pBVR3, 35S::cBVR1, M0062/UASBVR, hy1-1, and hy2-1 have significantly longer roots than their cognate WT parent when grown in the presence of 20 µM MeJA (Fig. 6B). 35S::pBVR3, 35S::cBVR1, hy1-1, and hy2-1 plants are ~73 to 100% longer than the WT parent grown under identical conditions; whereas M0062/UASBVR seedlings with root-specific phytochrome deficiency are ~21% longer than roots of C24 (Fig. 6B). The response for seedlings with root-localized phytochrome deficiencies is similar to that observed for characterized JA insensitive mutants, jar1 and myc2, which are ~100% and 61% longer than the Col-0 WT parent, respectively (Fig. 6B). This observation agrees...
with prior results for *jar1* (Staswick et al., 1992; Staswick et al., 2002; Lorenzo et al., 2004) and *myc2* (Lorenzo et al., 2004; Yadav et al., 2005; Gangappa et al., 2010).

As the root lengths of lines with root-localized BVR expression were already longer than the WT parents in the absence of JA (Fig. 2), we measured the relative JA sensitivity of these lines by calculating the ratio of the root length for a particular line in the absence of exogenous MeJA treatment to root length in the presence of JA. We determined that a line exhibiting root-localized, plastid-targeted BVR accumulation had a lower relative JA sensitivity than WT: 3.45-fold longer roots for 35S::pBVR3 in the absence of JA as compared to 4.75-fold longer roots for the No-0 WT parent (Table 1). This difference represents a reduction of ~27% in sensitivity to JA treatment in the 35S::pBVR3 line. The roots of the M0062/UASBVR line were 4.46-fold longer in the absence of added MeJA as compared to the lengths of seedlings grown in the presence of MeJA. This result represents a reduction of ~7% in sensitivity to exogenous JA relative to the C24 control. By comparison, the *hy1-1* mutant exhibited a ~50% reduction in sensitivity to JA, whereas JA sensitivity was reduced by ~37% for the *hy2-1* mutant relative to C20 WT. The results for lines with phytochrome deficiencies in roots are less than the ~67% and 49% reductions in JA sensitivity calculated for the *jar1* and *myc2* mutants relative to Col-0 WT, respectively. Notably, the 35S::cBVR1 line, which exhibits cytosolic BVR expression, was at least as sensitive to JA-mediated inhibition of root elongation as No-0 WT: the 35S::cBVR1 line exhibited 4.95-fold longer roots in the absence of MeJA than in the presence of 20 µM MeJA (Table 1), though the roots were significantly longer than No-0 WT both with and without MeJA treatment. The CAB3::pBVR lines, which exhibited marginally longer roots than the WT, showed no reduction in JA sensitivity (Table 1). The observance of longer roots for 35S promoter-driven BVR lines is apparent not only at 20 µM MeJA, but throughout a range of MeJA concentrations (Supplemental Fig. S2). Notably, the No-0, Col-0, and C24 ecotypes appear to be less sensitive to JA than the C20 ecotype: No-0, Col-0, and C24 roots are ~4.8X longer in the absence of JA than when grown in the presence of 20 µM JA, whereas C20 roots are 5.76X longer in the absence of JA (Table 1).
Expression of JA-inducible genes is impaired in lines with root-localized phytochrome deficiencies

To determine whether the JA-hyposensitive phenotype of lines with BVR-induced phytochrome deficiency in roots is correlated with a molecular phenotype in these lines, we investigated the expression of JA-inducible marker genes—*OPDA-REDUCTASE 3* (*OPR3*) and *VEGETATIVE STORAGE PROTEIN 1* (*VSP1*)—using quantitative reverse transcription PCR (qPCR) analyses. *OPR3* encodes a JA-biosynthetic enzyme, whereas *VSP1* is a JA-responsive marker gene. In qPCR analyses using RNA extracted from whole seedlings, we determined that all promoter-driven BVR lines exhibited reduced expression of *OPR3* independent of externally applied MeJA, with the 35S::cBVR1 line exhibiting the greatest reduction relative to No-O WT levels (Fig. 7). However, the gene expression in these lines was still highly sensitive to applied MeJA, i.e. all lines showed 6- to 7-fold greater expression of *OPR3* when 20mM MeJA was supplied (Fig. 7). The root-specific M0062/UASBVR line exhibited only minor differences in *OPR3* expression relative to C24 (Fig. 7). Notably, chromophore-deficient *hy1* and *hy2* mutants did not show as severe defects in *OPR3* expression, i.e., only *hy1* exhibited a 12% reduction relative to C20 WT in the presence of JA (Fig. 7). The *myc2* mutant exhibited slight reductions in *OPR3* expression independent of MeJA treatment (Fig. 7). The *jar1* mutant showed a minor reduction in *OPR3* expression in the presence of MeJA, similar to prior observations obtained using Northern blot analyses (Chung et al., 2008; Koo et al., 2009).

When assessing expression of *VSP1*, we observed that all lines exhibited extremely low levels of *VSP1* expression in the absence of MeJA (Fig. 7). In the presence of 20mM MeJA, all BVR lines, with the exception of the root-specific M0062/UASBVR line, exhibited reduced *VSP1* expression relative to the cognate WT (Fig. 7). The root-specific M0062/UASBVR line exhibited ~ 50 % greater expression of *VSP1* under these conditions (Fig. 7). *hy1* and *hy2* mutants exhibited increased *VSP1* expression independent of MeJA treatment as previously observed (Zhai et al., 2007). The *jar1* and *myc2* mutants showed reduced expression of *VSP1* independent of MeJA treatment (Fig. 7), which has been previously noted for *jar1* (Tuominen et al., 2004; Gangappa et al., 2010).
DISCUSSION

The merits of studying phytochrome responses in roots, particularly for seedlings and plants grown on agar media, which allows direct exposure of the entire body of plants to light, has been debated. However, the growth habits of Arabidopsis in shallow soils and highly disturbed environments and the recognition that light penetrates the upper layers of soil increase the likelihood that Arabidopsis roots are exposed to light and exhibit light-dependent responses in natural environments. Very early studies indicate a role for phytochrome in specific aspects of root development in pea (Furuya and Torrey, 1964), beans (Jaffe, 1970), and Convolvulus arvensis (Tepfer and Bonnett, 1972). Furthermore, recent results demonstrate that phyB mutant Arabidopsis plants grown on soil showed a similar defect in the initiation of lateral roots, as when the phyB mutant seedlings were grown on agar plates (Salisbury et al., 2007). Therefore, the impact of light perception and subsequent signaling by phytochromes can occur in more natural growth conditions, just as when seedlings are grown on plates.

Root-localized phytochromes contribute to tissue-specific control of root development

Phytochromes impact root development in Arabidopsis. We initiated in planta experiments to determine at the molecular level whether roots are the site of light perception for this response. GAL4 enhancer-trap lines are T-DNA insertion lines with diverse expression patterns of the yeast transcription factor, GAL4, whose expression depends on the presence of native genomic enhancer sequences. The GAL4-responsive mGFP5 gene marks the expression pattern mediated by genomic enhancers in green fluorescence (Haseloff, 1999; Laplaze et al., 2005). The BVR gene under control of the Upstream Activation Sequence (UAS) element is silently maintained in the absence of GAL4 in the UASBVR parent. Progeny from genetic crosses between a UASBVR transgenic line and a root-specific GAL4 enhancer trap line exhibited root-specific accumulation of the BVR protein (Fig. 3G). These lines did not display any light-
dependent changes in hypocotyl elongation, indicating that phytochrome function in shoots is not impacted in the M0062/UASBVR line (Fig. 5). However, we observed distinct growth phenotypes in lines that exhibit root-localized BVR accumulation, i.e., 35S::pBVR, 35S::cBVR1, and M0062/UASBVR lines, as compared to lines that lack root-localized BVR expression, i.e., No-0 WT, CAB3::pBVR, and MER15::pBVR lines (Fig. 2). Roots for lines with root-localized, BVR-induced phytochrome deficiencies are highly significantly different in regards to length than roots of seedlings of WT or parental lines under Wc illumination (Fig. 2).

Notably, roots in lines with root-localized BVR accumulation also are longer under Bc and Rc illumination (Fig. 4A and 4B). These findings suggest that R and B light are perceived directly by roots and subsequently effect root elongation. A tissue-specific role for the cry1 and cry2 proteins in roots was not supported in a prior study (Canamero et al., 2006), thus our data indicate a role for phyA in roots in the tissue-specific regulation of root elongation under Bc illumination. On the contrary, root-specific phytochrome depletion had little effect on root elongation under FRc (Fig. 4C). However, phyA stimulates root elongation as apparent from seedlings lacking phyA throughout the whole organism (Fig. 4C; Kurata and Yamamoto, 1997; Shin et al., 2010). Thus, phyA may impact root elongation largely from shoot-derived signals. In support of this suggestion, FR light is the wavelength most effectively conducted axially from shoots to roots (Sun et al., 2005) and a prior study, though limited to arrays with 7,000 elements, identified no FR-regulated genes locally in dark-adapted roots exposed to FR (Sato-Nara et al., 2004). Considering the results for Bc, Rc and FRc, we have demonstrated a root-specific role for phytochromes in the photoregulation of root elongation under distinct light conditions in the absence of altered phytochrome responsiveness in shoots in the M0062/UASBVR line.

As the 35S::pBVR3 line exhibits plastid-localized BVR expression, whereas 35S::cBVR1 and M0062/UASBVR lines exhibit cytosolic BVR expression, the observed phenotypes are likely associated with ΦΦ or phytochrome deficiency, as opposed to changes in plastid metabolism that could be correlated with plastid-localized BVR expression in 35S::pBVR lines (Franklin et al., 2003). Thus, the elongated roots of BVR lines exhibiting root-localized phytochrome deficiencies under Wc, Bc and Rc likely
represent the disruption of a phytochrome-dependent phenotype, e.g., the noted R-dependent inhibition of root elongation (Correll and Kiss, 2005) or disruptions in the co-action of phytochromes and cryptochromes (Ahmad and Cashmore, 1997; Guo et al., 2001; Usami et al., 2004). Furthermore, the occurrence of marginally longer roots in CAB3::pBVR2 relative to No-0 WT under Wc (Fig. 2B), and comparative analyses of root-specific vs. constitutive phytochrome deficiencies under distinct wavelengths of light (Fig. 4), indicate that shoot-localized phytochromes exhibit some long-distance control over root elongation. Previous results have demonstrated that shoot-derived signals impact root development in Arabidopsis (Salisbury et al., 2007). Ultimately, the reduced response observed for lines with either root-specific or mesophyll-specific BVR accumulation relative to constitutive BVR accumulators suggests that mesophyll-localized, or shoot-derived, phytochromes contribute to long distance regulation of root elongation, particularly under FR light as the root-specific BVR line did not differ significantly from WT under these conditions (Fig. 4C). However, these results provide strong evidence that root-localized phytochromes contribute locally to the photoregulation of root development in Arabidopsis.

Alterations in JA sensitivity are correlated with phytochrome-chromophore deficiency in roots

JA is known to inhibit germination and root elongation in Arabidopsis (Staswick et al., 1992). Different Arabidopsis ecotypes exhibit distinct sensitivities to treatment with JA (Table 1; Rao et al., 2000; Matthes et al., 2008). Of the ecotypes tested in our study, the C20 ecotype appears to be more sensitive to treatment with MeJA than any other ecotype (Table 1). 35S::pBVR3 and M0062/UASBVR lines with induced phytochrome-chromophore deficiency in roots exhibit reduced JA sensitivity in root inhibition assays, whereas the roots of plants lacking root-localized phytochrome chromophore depletion largely exhibit WT phenotypes in these assays (Table 1). The fact that only specific lines exhibiting BVR accumulation in roots—i.e., either constitutive 35S::pBVR3 or root-specific, enhancer-trap BVR expression—exhibit significant reduction in sensitivity to JA treatment relative to WT provides evidence that PΦB or
phytochrome deficiency itself in the root contributes significantly to light-dependent, JA-responsive elongation phenotypes. Though shoot-specific phytochromes can impact root elongation through inter-organ signaling as observed for the CAB3::pBVR2 line (Fig. 2), which exhibits shoot-specific phytochrome deficiency (Warnasooriya and Montgomery, 2009), root-localized phytochromes are most important for JA sensitivity as the CAB3::pBVR2 line has no alternation in JA sensitivity (Table 1).

Chromophore-deficient mutants hy1-1 and hy2-1 exhibited disparate responses in the absence of JA, with hy1-1 being shorter and hy2-1 being longer than the identically treated WT parent (Fig. 2). In the presence of JA, both lines were longer than the WT, exhibiting reduced sensitivity to JA (Fig. 6 and Table 1). This result observed for hy1 is distinct from what was previously reported for hy1-100 (Zhai et al., 2007). The hy1-100 allele exhibited shorter roots than its WT parent, although the responsiveness to JA was similar to that of the WT (Zhai et al., 2007). Our distinct observation may result from ecotypic differences or differences in growth conditions. In this regard, there are known ecotypic differences in the response to JA responsiveness (Table 1; Rao et al. 2000; Matthes et al., 2008) and root elongation responses in light-grown seedlings (Supplemental Fig. S1; Beemster et al. 2002; Passardi et al., 2007). In our studies, the hy1-1 mutant response resembles the JA-insensitive mutants jar1 and myc2 at the phenotypic level: shorter roots than WT in the absence of MeJA and longer roots in the presence of exogenously applied MeJA. By contrast, the hy2-1 mutant is more similar to other lines with root-localized phytochrome chromophore deficiency. These lines all have longer roots than WT independent of JA treatment and have reduced sensitivity to JA. Notably, 35S::cBVR1 is unique amongst the tested lines with root depleted PΦB, as it does not exhibit a significant difference in its sensitivity to JA (Table 1). This line has significantly longer roots than WT in the absence or presence of JA (Figs. 2 and 5), but shows a relative response to JA that is at least equal to that observed for the WT parent (Table 1).

Root-specific phytochrome deficiency alone was correlated with an increased level of expression of JA-responsive gene VSP1 in the presence of exogenously applied MeJA among BVR lines (Fig. 7). It has been shown that a repressor of JA signaling is regulated by phyA in shoots, but not in roots of Arabidopsis (Robson et al., 2010). Thus,
phyA can regulate distinct components that impact JA signaling in a tissue-specific manner. In comparison to root-specific BVR expression, 35S-promoter-driven BVR lines exhibited reduced levels of expression of VSP1 and OPR3 genes in response to MeJA treatment (Fig. 6). The lower levels in BVR lines, other than the root-specific line, suggest that BVR lines have lower JA biosynthetic capacity. These results are somewhat complicated, however, by the fact that light and MeJA synergistically impact VSP1 expression (Berger et al., 1995). Recently, it has been determined that phyA is required for VSP1 induction in the presence of JA (Robson et al., 2010). In BVR lines, both light signaling and JA responsiveness are impacted and thus the combined impact of PΦB or phytochrome deficiency and JA treatment on VSP1 expression in these lines is likely complex.

The disparate results observed in regards to the regulation of JA-associated genes in the root-specific BVR line relative to other BVR lines may be associated with a strictly localized degradation of the phytochrome chromophore or lack of phytochrome in the M0062/UASBVR line. This localized disruption of PΦB accumulation does not appear to result in a major impact on JA biosynthesis in the whole seedlings, which were used for RNA isolation for the gene expression studies. Notably, however, the result observed for the M0062/UASBVR line strongly implicates a need for phytochromes or the phytochrome chromophore specifically in roots for a WT, JA-mediated root inhibition response. Taken together, these data support a molecular link between phytochrome and JA signaling, and further demonstrate that these pathways interact in roots themselves. These results correspond strongly with recent results showing that JA perception by the root itself is required for inhibition of root growth in A. thaliana, as application of the hormone to roots, but not leaf application, led to decreased root growth (Schmidt et al., 2010). Furthermore, the results provide additional support for a tissue-specific role of phyA in regulating JA responses. Prior work demonstrated that JA-mediated responses in the shoot and in response to wounding are not impacted in a phyA mutant; whereas functional phyA and root growth inhibition in response to JA are definitively linked (Robson et. al, 2010). Our analyses further implicate root-localized phytochromes as the pool important for this link.
In summary, Arabidopsis lines that exhibit root-localized BVR accumulation have defects in the photoregulation of root elongation. Specific lines with root-localized BVR expression, including 35S::pBVR3 and M0062/UASBVR, also exhibit reduced sensitivity to the plant hormone JA. These results provide evidence that root-specific phytochrome chromophore, or root-localized phytochromes themselves, are critical for apposite photoregulation of root elongation and impact JA sensitivity.

MATERIALS AND METHODS

Plasmid Constructions

We isolated the pUAS1380-BVR (hereafter UASBVR) construct using the following method: the full-length BVR coding region was cloned using primers UASBVR_S (CGTCTAGAATGGATGCGGAGCCAAAG) and UASBVR_AS (CGAGATCTTTACTTCTCGTGGCAAAAG) with introduced XbaI and BglII restriction sites (underlined), respectively. The BVR coding region was PCR-amplified using pASK-FLBVR (BL Montgomery and JC Lagarias, unpublished data) as a template and the resulting PCR product restricted with XbaI and BglII enzymes (New England Biolabs). The digested PCR product was ligated to the similarly digested pUAS1380 plant transformation vector using a TaKaRa DNA Ligation Kit Ver. 2.1 (Takara Bio U.S.A., Inc.). The integrity of the plasmid was confirmed using restriction digestion and DNA sequencing analyses.

Plant Transformation and Materials

Wild-type (WT) Arabidopsis ecotype C24 plants were transformed with the UASBVR construct using standard methods for Agrobacterium-mediated floral dip as previously described (Clough and Bent, 1998). Antibiotic selection of putative UASBVR transformants was performed in 100- × 25-mm Petri dishes on media containing 1X Murashige and Skoog salts (MS salts; Caisson Laboratories), 0.8% (w/v) Phytablend (Caisson Laboratories), 1% (w/v) sucrose, and 50 μg/mL kanamycin, adjusted to pH 5.7.
with KOH. PCR-based screening was performed to isolate a homozygous UASBVR transformant, UASBVR1. T3 plants of UASBVR1 were crossed with the M0062 enhancer trap line, which exhibits root-specific GFP accumulation (Haseloff, 1999), and F1 progeny were planted on kanamycin for selection. The genotyping of F1 seedlings was performed with BVR- (forward, 5′–GGCTGAGGGACTTGAAGGATCCAC–3′, reverse, 5′– CACTTCTTCTGGTGAGCAAAGCTTC–3′) and GAL4-specific primers (forward, 5′–AGTGTCTGAAGAACAACTGGGAG–3′, reverse, 5′–CGAGTTTGGAGCAGATGTTTACC–3′). Thermal cycling conditions were 95°C for 2 min, 40 cycles of 95°C for 1 min, 60°C for 1 min (BVR-specific primers) or 58°C for 1 min (GAL4-specific primers), 72°C for 1 min, followed by 72°C for 5 min and a hold at 4°C. F1 seedlings that were identified as positive with both primer sets were transferred to soil and propagated to subsequently obtain F3 seeds of a cross between M0062 and UASBVR—i.e., M0062/UASBVR—that were used in our assays.

*A. thaliana* ecotypes C24, Col-0, and C20 were used as WT and were obtained from the Arabidopsis Biological Resource Center (ABRC; http://www.biosci.ohio-state.edu/pcmb/Facilities/abrc/abrchome.htm; (Alonso et al., 2003). Enhancer trap line M0062, and chromophore-deficient mutants hy1-1 and hy2-1 were obtained from ABRC. No-0 WT and transgenic BVR lines—35S::pBVR3, 35S::cBVR1, CAB3::pBVR2, and MERI5::pBVR1—were previously described (Montgomery et al., 1999; Warnasooriya and Montgomery, 2009). Mutants jar1-1 (Staswick et al., 2002) and myc2 (Lorenzo et al., 2004) were previously described. All wild type and mutant seed stocks used in the experiments described here were derived from plants grown at the same time in the same controlled environment growth chamber under white light at 100 μmol m⁻² s⁻¹ under daily cycles of 16 h of light and 8 h of darkness at 20 °C.

**Plant Growth Conditions**

Seeds were sterilized with 35% (v/v) commercial bleach and 0.025% (v/v) SDS solution, then rinsed with ultrapure water (Milli-Q, Millipore) as previously described (Warnasooriya and Montgomery, 2009). Seeds were planted in 100- × 100- × 15-mm
square Petri dishes on media containing 1X MS salts, 0.8% (w/v) Phytablend, 0.05% (w/v) 4-Morpholineethanesulfonic acid (MES; Sigma), and 1% (w/v) sucrose, adjusted to pH 5.7 with KOH, and with or without 20 μM methyl jasmonic acid (MeJA; Sigma). Imbibing seeds were cold-stratified at 4°C for 3 d in darkness. Plates with stratified seeds were kept vertically in a humidity-controlled chamber under defined light conditions for 10 d at 22°C.

**Light Sources**

For continuous white light (Wc) growth, seeds on Phytablend media were grown in a Percival CU-36L5 Tissue Culture Chamber under cool white fluorescent illumination of 100 μmol m⁻² s⁻¹. Continuous red (Rc), blue (Bc) and far-red (FRc) sources were those previously described (Warnasooriya and Montgomery, 2009). We measured Wc, Rc, and Bc fluence rates using a LI-250A Light Meter (LI-COR) equipped with a quantum sensor (LI-COR) and FRc were measured using a StellarNet EPP2000 spectroradiometer (Apogee Instruments).

**Whole–Mount Immunohistochemical Analysis**

Three-and-a-half-d-old seedlings grown in vertically placed plates as described above were subjected to whole-mount *in situ* protein localization to visualize proteins in root tips, lateral roots, and embryos, as previously described with limited modifications (Sauer et al., 2006). Seedlings were treated with the paraformaldehyde-based fixative solution for 30 min followed by washing with 1X PBS for 2×10 min and with sterile water for 2×5 min. Fixed seedlings were mounted on Poly-Prep slides (Sigma) in a droplet of water and were air dried for 2.5 h at room temperature. Cell walls were digested with 2% (w/v) Driselase (Sigma) in 1X PBS for 30 min at 37°C. Slides were washed with 1X PBS for 3×10 min. Tissues were permeabilized with 3% (v/v) IGEPAL CA-630 containing 10% (v/v) dimethylsulfoxide for 1 h at room temperature, followed by washing with 1X PBS for 4×10 min. Blocking with 3% (v/v) bovine serum albumin fraction V (Roche) was carried out for 1 h at room temperature. Fixed and permeated
seedlings were incubated with rabbit anti-BVR antibody (QED Bioscience Inc.) at 1:2000, 1:3000 or 1:4000 dilution as indicated in 1X PBS or with 1X PBS alone for control samples overnight at 4°C. Excess primary antibody was removed by washing slides with 1X PBS for 3×10 min. Following incubation with the primary antibody and washing, seedlings were incubated with goat anti-rabbit IgG (H+L) conjugated to HiLyte Plus™ 555 (AnaSpec) at 0.005 mg/mL dilution in 1X PBS for 6 h at 37°C. To remove excess secondary antibody, slides were washed with 1X PBS for 4×10 min. Drops of antifade mountant medium (Citifluor) were placed on treated seedlings and covered with cover slips. Slides were stored overnight at 4°C before imaging. Root tips of seedlings were imaged on an inverted Axiovert 200 Zeiss LSM 510 Meta confocal laser scanning microscope (Carl Zeiss MicroImaging) using differential interference contrast (DIC) optics and fluorescence excitation/emission filters. A 20×0.75 Plan Apochromat objective lens was used for imaging. DIC imaging was performed using the 543-nm laser. Fluorescence from the secondary antibody was collected using a 543-nm laser for excitation and a 560 – 615 nm band pass filter for emission. Images were acquired using the LSM FCS Zeiss 510 Meta AIM imaging software.

**Root Length Measurements**

Prior to conducting detailed root length analyses at 10 days, we conducted preliminary observations daily between 3 and 11 days after transferring seeds to experimental growth chambers and observed the same relative differences in root length throughout the observation period (see Supplemental Fig. S1 for representative images of WT lines at 3, 5, 8 and 10 days). As the roots of seedlings grown in the presence of JA are shorter, we used a longer time point of 10 days for replicative root length assays. Plates containing 10-d-old seedlings were scanned and plant images were used to quantify root lengths in endpoint length measurement assays using ImageJ software (NIH). Root length assays were repeated at least 3 times. Two-tailed, unpaired Student’s t-test (for normally distributed data) or Mann-Whitney U-test (for non-normal data distributions) was performed to compare the means of root lengths.
Hypocotyl Inhibition Assays

Sterilized seeds of No-0 WT, 35S::cBVR1, C24 WT, and M0062/UASBVR were cold-stratified at 4 °C for 3 days in darkness. Plates were kept in a humidity-controlled chamber with Bc of 30 µmol m⁻² s⁻¹, Rc illumination of 50 µmol m⁻² s⁻¹, FRc illumination of 10 µmol m⁻² s⁻¹, or in darkness for 7 days at 22 °C. Seedlings were scanned and plant images were used to quantify hypocotyl lengths using ImageJ software (NIH). The hypocotyl inhibition assay was repeated 3 times. Percentage dark length and standard deviations of percentage dark length are reported. Two-tailed, unpaired Student’s t-test was performed to compare the percentage dark length of hypocotyls of transgenic lines relative to cognate WT seedlings, except for C24 WT, and M0062/UASBVR grown in Rc and FRc, where two-tailed, unpaired Mann-Whitney U-test was performed to compare the percentage dark length of hypocotyls of M0062/UASBVR to that of C24 WT.

RT-PCR Analyses

Real-time quantitative RT-PCR (qPCR) was performed to quantify the levels of transcripts of a JA biosynthetic gene, 12-OPDA REDUCTASE3 (OPR3; At2g06050), and a JA-inducible marker gene, VEGETATIVE STORAGE PROTEIN1 (VSP1; At5g24780). Seeds of No-0 WT, 35S::pBVR3, 35S::cBVR1, CAB3::pBVR2, MERI5::pBVR1, Col-0 WT, jar1, myc02-05, C24 WT, M0062/UASBVR, C20 WT, hyl1-1 and hy2-1 were planted in 245- x 245- x 18-mm square petri dishes on MS media with or without 20 μM MeJA (Sigma). Imbibing seeds were cold-stratified at 4 °C for 3 days in darkness. Plates were kept vertically in a humidity-controlled chamber with Wc illumination of 100 µmol m⁻² s⁻¹ for 10 days at 22 °C. 10-d-old whole seedlings were quickly (< 1 min) harvested and immediately frozen in liquid nitrogen. Using RNeasy® Plant Minikit (Qiagen) including on-column DNase treatment (Qiagen), total RNA was isolated according to manufacturer’s instructions. Quantity of the RNA was analyzed by spectrometry (NanoDrop1000, Thermo Scientific). First-strand cDNA synthesis was performed using the Reverse Transcription System (Promega) with random primers according to the manufacturer’s instructions using a 20 µL reaction volume. The incubation times of first-
strand cDNA synthesis with total RNA of 0.2 µg was (1) 10 min at room temperature, (2) 1 h (instead of 15 min) at 42 °C, (3) 5 min at 95 °C and (4) 5 min at 4 °C. The cDNA reaction mixture was diluted forty-fold with Nuclease-Free Water, and 4 µL of the diluted cDNA product was used as template in a 10 µL qPCR reaction using the Applied Biosystems FAST 7500 real-time PCR system in FAST mode with Fast SYBR® Green Master Mix (Applied Biosystems) according to manufacturer’s instructions. For transcript analysis, annealing/extension temperature was 60 °C for both the OPR3 and VSP1 primer sets. Reactions were performed in triplicate and products were checked by melting curve analysis. The abundance of transcripts was analyzed using the ddCt method based on relative quantification, normalizing to the reference transcript UBC21 (At5g25760). All qPCR experiments were repeated with three independent biological replicates. All qPCR procedures and analysis conform to MIQE guidelines (Bustin et al., 2009).

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FIGURE LEGENDS

**Figure 1.** Whole-mount immunolocalization of BVR protein accumulation in roots of wild-type and promoter-driven BVR seedlings. No-0 WT (A, F), 35S::pBVR3 (B, G), 35S::cBVR1 (C, H) CAB3::pBVR2 (D, I), and MERI5::pBVR1 (E, J) seedlings were grown at 22°C on Phytablend medium containing 1% (w/v) sucrose for ~4 d under Wc illumination of 100 μmol m⁻² s⁻¹. Seedlings were incubated with anti-BVR primary antibody at a 1:4000 dilution, except for 1:3000 dilution for 35S::cBVR1. Upper image is fluorescence and lower image is DIC for each seedling. Each image is a representative slice from a Z-series with 0.5 μm interval size and was captured using 543 nm laser excitation with a 20× lens objective. Fluorescence images were collected using a 560 – 615 nm band pass filter. Bar, 50 μm.

**Figure 2.** Photomorphogenesis and root elongation responses of wild-type, phytochrome chromophore deficient and jasmonic acid (JA)-insensitive mutants grown under continuous white illumination. No-0 wild-type (WT), 35S::pBVR3, 35S::cBVR1, CAB3::pBVR2 (CAB-2), MERI5::pBVR1 (MERI-1), C24 WT, F3 seedlings of a M0062×UASBVR cross (M0062/UASBVR), C20 WT, hy1-1, hy2-1, Col-0 WT, jar1, and myc2 lines were grown at 22°C on Phytablend medium containing 0.8% (w/v) sucrose with no added methyl jasmonate for 10 d under Wc illumination of 100 μmol m⁻² s⁻¹. (A) Images of seedlings. Bar, 1 cm. (B) Bars represent the mean (+S.D.) of root lengths in mm (n ≥ 10 for each of six independent experiments). For statistical significance tests comparisons were made relative to cognate wild-type lines: a, p<0.0001; b, p<0.01; and c, p<0.05. For information on the range of seedling lengths observed for each line, see summarized frequency distribution data (Supplemental Table S1).

**Figure 3.** Whole-mount immunolocalization of BVR protein accumulation in roots of wild-type and enhancer-trap BVR seedlings. C24 WT (A–D) and F3 seedlings of a M0062×UASBVR cross (M0062/UASBVR) (E–H) were grown at 22°C on Phytablend medium containing 1% (w/v) sucrose for ~4 d under Wc illumination of 100 μmol m⁻² s⁻¹. Fluorescence images of seedlings incubated without (A, E) or with (C, G) anti-BVR
primary antibody (Ab) at a 1:2000 dilution are shown. DIC images (B, D, F, H) are shown for each seedling. Each image is a representative slice from a Z-series with 0.5 µm interval size, captured using 543 nm laser excitation with a 20× lens objective. Fluorescence images were collected using a 560 – 615 nm band pass filter. Bar, 50 µm.

**Figure 4.** Light-dependent root elongation responses of wild-type, phytochrome chromophore deficient and phytochrome apoprotein mutants. No-0 wild-type (WT), 35S::pBVR3, 35S::cBVR1, C24 WT, F3 seedlings of a M0062×UASBVR cross (M0062/UASBVR), C20 WT, hy1-1, hy2-1, Col-0 WT, phyA and phyB lines were grown on Phytablend medium containing 1% (w/v) Suc for 10 d at 22°C under blue continuous (Bc) light of 30 µmol m⁻² s⁻¹, red continuous (Rc) light of 50 µmol m⁻² s⁻¹, and far-red continuous (FRc) light of 10 µmol m⁻² s⁻¹. (A, B, C). Bars represent the mean (±S.D.) of root lengths in mm (n≥10 for each of three independent experiments). For statistical significance tests comparisons were made relative to cognate wild-type lines: a, p<0.001; b, p<0.01; and c, p<0.05.

**Figure 5.** Photomorphogenesis and mean hypocotyl lengths of wild-type and BVR-expressing seedlings. No-0 wild-type (No-0 WT), 35S::cBVR1, C24 wild-type (C24 WT) and M0062/UASBVR lines were grown on Phytablend medium containing 1 % (w/v) Suc for 7 d at 22 °C under blue continuous (Bc) light of 30 µmol m⁻² s⁻¹, red continuous (Rc) light of 50 µmol m⁻² s⁻¹, and far-red continuous (FRc) light of 10 µmol m⁻² s⁻¹. (A, B, C) Images of seedlings. Bars, 1 cm. (D) Bars represent mean (± SD) of hypocotyl lengths measured for 10 or more hypocotyls in each of three independent experiments. (A) Bc, blue bars; (B) Rc, red bars; and (C) FRc, dark red bars.

**Figure 6.** Photomorphogenesis and root elongation response of wild-type, phytochrome chromophore deficient and jasmonic acid (JA)-insensitive mutants grown under continuous white illumination with added methyl jasmonate. No-0 wild-type (WT), 35S::pBVR3, 35S::cBVR1, CAB3::pBVR2 (CAB-2), MERI5::pBVR1 (MERI-1), C24 WT, F3 seedlings of a M0062×UASBVR cross (M0062/UASBVR), C20 WT, hy1-1, hy2-1, Col-0 WT, jar1, and myc2 lines were grown at 22°C on Phytablend medium.
containing 0.8% (w/v) sucrose with 20 µM methyl jasmonate for 10 d under Wc illumination of 100 µmol m⁻² s⁻¹ (A) Images of seedlings. Bar, 1 cm. (B) Bars represent the mean (±S.D.) of root lengths in mm (n≥10 for each of six independent experiments). For statistical significance tests comparisons were made relative to cognate wild-type lines: a, p<0.0001 and b, p<0.01. For information on the range of seedling lengths observed for each line, see summarized frequency distribution data (Supplemental Table S1).

Figure 7. Relative expression levels of jasmonic acid-associated genes in wild-type, BVR-expressing and mutant seedlings. Expression of jasmonic-acid (JA) biosynthetic gene OPR3 (A) and JA-responsive marker gene VSP1 (B) in 10-d-old whole seedlings grown at 22ºC on Phytablend media with or without 20 µM methyl jasmonate under Wc illumination of 100 µmol m⁻² s⁻¹. Quantitative PCR was conducted using RNA from No-0 wild-type (No-0 WT), 35S::pBVR3, 35S::cBVR1, CAB3::pBVR2, MERI5::pBVR1, C24 wild-type (C24 WT), M0062/UASBVR, C20 wild-type (C20 WT), hy1-1, hy2-1, Col-0 wild-type (Col-0 WT), jar1, and myc2 lines. Expression of UBC21 (At5g25760), which is a control gene encoding an ubiquitin conjugating enzyme, was analyzed as a reference. Bars, black bars, -JA and white bars, + JA (20 µM). Quantification by qPCR was performed with 3 independent experiments. Fold difference (Fold diff.) for levels of transcript accumulated in test line relative to the cognate WT line is shown below each graph.
Table 1. Fold-difference values for root lengths determined for seedlings with respect to cognate wild-types grown in the absence vs. presence of methyl jasmonate (MeJA). No-0 wild-type (No-0 WT), 35S::pBVR3, 35S::cBVR1, CAB3::pBVR2, MERI5::pBVR1, C24 wild-type (C24 WT), M0062/UASBVR, C20 wild-type (C20 WT), hy1-1, hy2-1, Col-0 wild-type (Col-0 WT), jar1, and myc2 seedlings were growth with or without added methyl jasmonate (MeJA). Fold-difference values for average root lengths for seedlings grown on 0 µM JA relative to 20 µM JA are indicated (n ≥ 10 for each of six independent experiments). The percent (%) reduction in fold difference of root lengths relative to the cognate WT was calculated.

| Plant line          | Fold difference of root length (-MeJA/+MeJA) | % reduction in responsiveness relative to WT |
|---------------------|----------------------------------------------|---------------------------------------------|
| No-0 WT             | 4.75                                         | -                                           |
| 35S::pBVR3          | 3.45                                         | 27.3                                        |
| 35S::cBVR1          | 4.95                                         | NA<sup>a</sup>                              |
| CAB3::pBVR2         | 4.74                                         | 0                                           |
| MERI5::pBVR1        | 4.23                                         | 11                                          |
| C24 WT              | 4.80                                         | -                                           |
| M0062/UASBVR        | 4.46                                         | 7                                           |
| C20 WT              | 5.76                                         | -                                           |
| hy1-1               | 2.82                                         | 51                                          |
| hy2-1               | 3.60                                         | 37.5                                        |
| Col-0 WT            | 4.85                                         | -                                           |
| jar1                | 1.62                                         | 67                                          |
| myc2                | 2.48                                         | 49                                          |

<sup>a</sup>NA, not applicable. The lengths of these seedlings were 0 to 4% longer than WT.
Supplemental Material

**Supplemental Table S1.** Frequency distribution analyses for root lengths of wild-type, phytochrome chromophore deficient and jasmonic acid-insensitive mutants under continuous white illumination. No-0 wild-type (No-0 WT), 35S::pBVR3, 35S::cBVR1, CAB3::pBVR2, MERI5::pBVR1, C24 wild-type (C24 WT), M0062/UASBVR, C20 wild-type (C20 WT), *hy1-1*, *hy2-1*, Col-0 wild-type (Col-0 WT), *jar1*, and *myc2* seedlings were grown with or without added methyl jasmonate (MeJA). Data represent root lengths in mm (n≥10 for each of six independent experiments).

| Plant Line         | 0 mM MeJA |                           | 20 mM MeJA |                           |
|--------------------|-----------|---------------------------|------------|---------------------------|
|                    | n         | min<sup>a</sup> | max<sup>b</sup> | median | n       | min  | max<sup>b</sup> | median |
| No-0 WT            | 91        | 4.001-5     | 64.001-65 | 34.001-35 | 97      | 1.001-2 | 13.001-14 | 6.001-7 |
| 35S::pBVR3         | 35        | 18.001-19   | 68.001-69 | 49.001-50 | 34      | 3.001-4 | 21.001-22 | 13.001-14 |
| 35S::cBVR1         | 33        | 28.001-29   | 69.001-70 | 62.001-63 | 32      | 3.001-4 | 19.001-20 | 11.001-12 |
| CAB3::pBVR3        | 32        | 14.001-15   | 52.001-53 | 39.001-41 | 28      | 2.001-3 | 14.001-15 | 7.001-9  |
| MERI5::pBVR1       | 36        | 11.001-12   | 47.001-48 | 38.001-39 | 36      | 1.001-2 | 13.001-14 | 7.001-8  |
| C24 WT             | 62        | 6.001-7     | 41.001-42 | 31.001-32 | 44      | 2.001-3 | 10.001-11 | 5.001-6  |
| M0062/UASBVR       | 36        | 6.001-7     | 50.001-42 | 36.001-33 | 33      | 2.001-3 | 14.001-15 | 6.001-7  |
| C20 WT             | 26        | 18.001-19   | 93.001-94 | 36.001-38 | 26      | 1.001-2 | 15.001-16 | 5.001-7  |
| *hy1-1*            | 33        | 3.001-4     | 65.001-66 | 33.001-34 | 26      | 4.001-5 | 21.001-22 | 12.001-13 |
| *hy2-1*            | 25        | 11.001-1    | 99.001-99 | 47.001-34 | 27      | 3.001-4 | 28.001-12 | 12.001-12 |
|       | 12 | 100 | 48 | 29 | 14 |
|-------|----|-----|----|----|----|
| Col-0 WT | 45 | 10.001-67.001-53.001-42 | 5.001-6 | 16.001-10.001-11 | 68 | 54 | 17 | 11 |
| jar1   | 32 | 6.001-7 | 58.001-35.001-30 | 2.001-3 | 43.001-23.001-59 | 36 | 44 | 24 |
| myc2   | 33 | 7.001-8 | 68.001-43.001-33 | 3.001-4 | 32.001-18.001-69 | 44 | 33 | 19 |

\(^a\text{min, minimum class interval (root length in mm) in frequency distribution}\)
\(^b\text{max, maximum class interval (root length in mm) in frequency distribution}\)
**Supplemental Figure S1.** Representative images showing root lengths of wild-type *Arabidopsis thaliana* ecotypes over time. No-0 wild-type (WT), C24 WT, C20 WT, and Col-0 WT lines were grown at 22°C on Phytablend medium containing 0.8% (w/v) sucrose for 3, 5, 8 and 10 d under continuous white illumination at a fluence rate of 100 μmol m⁻² s⁻¹. Bar, 1 cm.

**Supplemental Figure S2.** Methyl jasmonate response curve for JA-mediated root growth inhibition in wild type and *BVR*-expressing lines. The impact of increasing concentrations of MeJA on the inhibition of root elongation was determined for No-0 WT (black bars), 35S::pBVR3 (gray bars), and 35S::cBVR1 (white bars) seedlings grown at 22°C on Phytablend medium containing 0.8% (w/v) sucrose with 0, 2.5, 5, 10, 15, or 20 μM methyl jasmonate for 10 d under continuous white illumination at a fluence rate of 100 μmol m⁻² s⁻¹. Bars represent the mean (±S.D.) of root lengths in mm (n≥10 for each of three independent experiments).
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