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Arabidopsis defense mutant ndr1-1 displays accelerated development and early flowering mediated by the hormone gibberellic acid

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Gibberellic Acid Mediates Accelerated Development and Early Flowering in the *Arabidopsis ndr1-1* Mutant

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Abstract

***NONRACE-SPECIFIC DISEASE RESISTANCE (NDR1*) is a widely characterized gene that plays a key role in defense against multiple bacterial, fungal, oomycete and nematode pathogens in plants. *NDR1* is required for activation of resistance by multiple NB and LRR-containing (NLR) protein immune sensors, and contributes to basal defense. The role of *NDR1* in positively regulating salicylic acid (SA)-mediated plant defense responses is well-documented. However, *ndr1-1* plants flower earlier and show accelerated development in comparison to wild-type (WT) Arabidopsis plants, suggesting that *NDR1* is a negative regulator of flowering and growth. Exogenous application of gibberellic acid (GA) further**

accelerates the early flowering phenotype in *ndr1-1* plants, while the GA biosynthesis inhibitor paclobutrazol attenuated the early flowering phenotype of *ndr1-1*, but not to WT levels, suggesting partial resistance to paclobutrazol and enhanced GA response by *ndr1-1*. Mass spectroscopy analyses confirmed that *ndr1-1* plants have 30-40% higher levels of GA_3 and GA_4, while expression of GA metabolic genes *GA2ox*, *GA3ox*, *GA20ox*, and major flowering regulatory genes *GI, CO*, *FT, LFY* and *AP1* are also altered in the *ndr1-1* mutant. Taken together, our discovery of crosstalk between phytohormone signaling and GA-regulated developmental and SA-regulated defense programs vis-à-vis the defense regulatory gene *NDR1* underscores the importance of elucidating these networks prior to manipulating them in crop plants.

Introduction

The *NONRACE-SPECIFIC DISEASE RESISTANCE* (*NDR1*) gene is a critical component of the plant immune system, and is necessary for the activation of appropriate defense responses against a wide array of plant pathogens, including the bacterial speck pathogen *Pseudomonas syringae*[1,2,3], the downy mildew pathogen *Perenospora parasitica*[1,4,5], the fungal vascular wilt pathogen *Verticillium spp.*[6,7], and the soybean root knot nematode *Heterodera glycines*[8]**.** *NDR1* is a glycosylphosphatidylinositol-anchored plasma membrane-localized protein that forms linkages with the cell wall, and is important for mediating electrolyte leakage[9,10,11,12]. Although *NDR1* has been widely characterized as a positive regulator of resistance gene-mediated defense responses, the biochemical function of *NDR1* is not fully defined and rarely has it been implicated in other plant processes apart from defense.

Though first discovered in *Arabidopsis thaliana*, homologs of *Arabidopsis NDR1* (Ath*NDR1*), including those from soybean, cotton, coffee and citrus are vital for defense[3,7,8,13,14]. In *Arabidopsis*, a loss-of-function mutation in *NDR1* conferred by a ~ 1.2 kb deletion in its single exon (*ndr1-1*) results in susceptibility to pathogens to which it is otherwise immune[2]. Even though multiple NB and LRR-containing proteins (NLRs), including the coiled-coil nucleotide-binding-site leucine-rich repeat (CC-NBS-LRR) type NLR-encoded genes *RPM1, RPS2,* and *RPS5* require *NDR1* for activation of plant defense responses against the bacterial effectors *avrRpm1*, *avrRpt2* and *avrPphB*, respectively[1,2,5,12,15], data indicating direct interaction of *NDR1* with these pathogen avirulence proteins is lacking. Despite the lack of evidence for a direct interaction between *NDR1* and NLR proteins, interaction between *RIN4* (an effector targeted protein guarded by *RPM1* and *RPS2*) and *NDR1* has been reported and this interaction is integral for resistance against specific bacterial effectors[16,17,18]. *NDR1* has also been implicated in hypersensitive responses (HR) initiated during incompatible plant-pathogen interactions in a salicylic acid (SA)-dependent manner, and *NDR1* function is associated with SA accumulation, systemic acquired resistance (SAR), and reactive oxygen species (ROS) accumulation following pathogen challenge[12].

Both biotic and abiotic stresses cause alterations in the normal transition to flowering in plants[22,23]. For example, the defense-associated plant hormone SA is known to delay flowering while ultraviolet light stress activates the transition to flowering in *Arabidopsis* in a SA-dependent manner[24]. Given that SA-mediated defense signaling is dependent on functional *NDR1*, and that key components of the SA defense pathway, including *NDR1*, are required for resistance against *V. longisporum*[6,7,21], it is not surprising that prior research efforts have noted a relationship between plant development and early flowering on Verticillium wilt symptom appearance[6,25,26,27]. However, the mechanism(s) underpinning these relationship(s) remain largely undefined.

The fungal genus *Verticillium* includes several soil-borne pathogens, which infect plant roots, and cause vascular wilt diseases in a variety of economically important crops worldwide[19,20]. *NDR1* was previously implicated in conferring resistance to Verticillium wilt in *Arabidopsis* and *Gossypium* (cotton), since plants lacking a functional *NDR1* were susceptible to the pathogen[6,7,21]. In the present work, we tested the hypothesis that *NDR1* plays a role in *Verticillium*-mediated alterations in the transition to flowering in *Arabidopsis*. We discovered that the genetic mutation in *ndr1-1* leads to de-repression of flowering in *Arabidopsis,* implying that *NDR1* is a negative regulator of flowering. Further, we provide evidence that the relief of negative regulation in *ndr1-1* mutant plants is mediated by an increased concentration of bioactive gibberellic acid (GA) and/or an enhanced response of GA-mediated signaling. We also demonstrate that major floral pathway regulatory genes that control the transition to flowering in *Arabidopsis* are up-regulated in the untreated *ndr1-1* background, underlying the early flowering phenotype observed in the mutant. Taken together, our discovery of the hitherto unknown role of *NDR1* in regulating developmental processes, including flowering time through GA response pathways, underscores the necessity of understanding the interplay between development and defense prior to the utilization of regulatory genes such as *NDR1* for enhancing disease resistance in crop plants through either traditional breeding approaches or genetic modifications.

Results

Verticillium infection accelerates flowering in *ndr1-1* plants

To examine the putative linkage between early flowering and defense responses, we measured flowering time after *Verticillium* infection in various *Arabidopsis* mutants lacking functional defense genes. These mutants included those defective in SA and ethylene-mediated defense

signaling pathways, including *eds16-1*, *npr1-1*, *etr1-1*, and *ndr1-1*[6]. In the loss-of-function mutant *ndr1-1*, multiple symptoms of Verticillium wilt, including accelerated flowering and a decrease in rosette size and bolt length are enhanced relative to these symptoms in the infected WT Col-0 plants. In accordance with our hypothesis, pathogen-challenged *ndr1-1* plants not only displayed enhanced susceptibility, as previously reported[6] to *Verticillium* strains (Fig. 1a), but also a significant acceleration in flowering time relative to the pathogen-treated control plants (Fig. 1b, c and d). Further, there was a significant decrease in the final main bolt length and number of siliques on the main bolt (Fig. 1e and f) as well as visible reduction in plant size with all three *Verticillium* strains tested (data shown in Fig. 1a for *V. longisporium* infection only).

Non-pathogen treated *ndr1-1* mutants display an early flowering phenotype

Subsequent analyses of *ndr1-1* plants together with the parental WT Col-0 plants in the absence of the pathogen revealed an early flowering phenotype in *ndr1-1* plants, indicating that the loss-of-function mutation in the *NDR1* gene leads to negative regulation of flowering responses in *Arabidopsis* (Fig. 2a). The *ndr1-1* mutant plants transitioned to the reproductive phase/flowering faster than the WT Col-0 plants as is evident from the appearance of the visible buds, subsequently bolting earlier (i.e., produced seed bearing primary inflorescence) with early appearance of open terminal flower, as well as exhibiting fewer rosette leaves than the WT Col-0 plants (Fig. 2d, e and f). This is indicative of faster progression through intrinsic developmental processes, even in the absence of any external signals/environmental cues (Fig. 2d and f). The average height of the bolts of the *ndr1-1* mutant plants when the first terminal flower opened was also significantly greater than the parental WT plants (Fig. 2h). A similar early flowering and faster bolting phenotype was observed in plants grown under short day conditions (Supplementary Figure 1), underscoring the

fact that the genetic lesion in *ndr1-1* mutant releases an inhibition on flowering, irrespective of the photoperiod and thereby suggests a role for GA in the regulation of flowering through an autonomous pathway in addition to known roles in the elongation of the stem/bolt[28,30,31].

Arabidopsis ndr1-1 mutants display accelerated development throughout the plant lifecycle

After the flowering phenotype was evaluated and scored, the plants were grown to maturity and the seeds collected. Plants were scored weekly for height of the main bolt as an indicator of growth until maturity, when all of the visible growth ceased and the plants completely senesced. More precisely, under our growth conditions all of the plants from *ndr-1-1* and WT Col-0 had flowered by the sixth week under long day conditions. Beginning at the fifth and sixth week of growth, *ndr1-1* plants that had earlier grown noticeably taller than the parental WT Col-0 plants, and henceforth displayed smaller increments of main bolt growth than the WT parents (Fig. 2a, b, c, g and i). Thus, at this later stage of growth, the *ndr1-1* plants displayed a slower incremental growth while WT Col-0 grew vigorously at the comparable stage, consistent with the progression of an overall faster intrinsic developmental program in *ndr1-1* compared with the WT parents. The more rapid *ndr1-1*-associated developmental program occurred without the input of additional biotic or abiotic trigger/signal. Although *ndr1-1* mutants were taller than the WT plants during early development due to their earlier and faster bolting (Fig. 2a), by the sixth week, WT plants were of equal height to the *ndr1-1* plants (Fig. 2c and i) and by the eighth week had surpassed the WT (Fig. 2d). Thus, at this later stage of growth, progression of an overall faster intrinsic developmental program in *ndr1-1* in comparison to the WT parents was evident. This rapid *ndr1-1*-associated developmental program occurs without the input of additional biotic or abiotic trigger/signal.

Although the silique setting occurred earlier in the *ndr1-1* plants, they set fewer total siliques on the main bolt as well as on the overall plant on average in comparison to WT Col-0 plants (Fig. 2j and k) and hence had correspondingly lower seed yield (Supplementary Figure 2a and b). These results indicate that the *ndr1-1* mutation not only leads to early flowering in *Arabidopsis* but also results in accelerated development, early completion of the life cycle, and reduced reproductive potential. This aberrant development was also apparent from the early induction of whole plant senescence, a tightly regulated developmental process in plants, in *ndr1-1* plants in comparison to WT Col-0 (Fig. 2a, b and c).

Genes promoting flowering are upregulated in *ndr1-1* plants

To begin to address the molecular basis of *ndr1-1* mutants-induced early flowering, expression of regulatory genes that control the transition to flowering under normal growth conditions[29,30,31] were studied. The levels of *GIGANTEA (GI)*, *CONSTANS (CO), FLOWERING LOCUS T (FT), SUPPRESSOR OF CONSTANS1 (SOC1), LEAFY (LFY)* and *APETALA1 (AP1)*, transcripts were markedly increased while that of the floral repressors *TERMINAL FLOWER 1 (TFL1)*, *SHORT VEGETATIVE PHASE1 (SVP1)* and *TEMPERANILLO 1 (TEM1)* were markedly down-regulated in the *ndr1-1* mutant plants relative to the wild type as early as eight days post-germination (dpg) (Fig. 3a).

Increased expression of gibberellic acid regulation and GA biosynthesis in *ndr1-1* plants

Transition to flowering in plants is induced by the sensing of many environmental cues, including day length, vernalization, hormones, and various biotic and abiotic stresses[22,23,24,29,30,31,32]. Among the phytohormones that have been shown to regulate floral

transition in the facultative long day *Arabidopsis* is the hormone Gibberellic Acid (GA)[30,31,32]. GA promotes flowering under non-inductive short day conditions as well as under long day conditions[30,33]. Since *ndr1-1* mutants exhibited accelerated flowering, increased bolt lengths, and later displayed a phenotype reminiscent of the *spindly* mutation (early flowering, spindly phenotype and negative regulator of the GA signaling) [28], we hypothesized that GA signaling might also be altered in *ndr1-1* plants.

To examine whether GA synthesis and regulation may be impacted in *ndr1-1* plants, we assessed the expression levels of GA biosynthetic or regulatory genes [32,33,34,35,36], in both *ndr1-1* and WT plants. Enhanced accumulation of transcripts of the GA biosynthetic genes *GA3Ox2* and *GA20Ox2* was observed in *ndr1-1* plants (Fig. 3b, right panel). There was also a concomitant decrease in the transcript levels of the GA catabolic genes *GA2Ox6* and *GA2Ox7*, which convert GA into an inactive form *in planta* [33,34,35](Fig. 3b, left panel).

The flowering phenotype could be the result of enhanced GA sensing in the *ndr1-1* mutant relative to the WT, a higher accumulation of GA in the *ndr1-1* mutant, or a combination of both. To determine if the early flowering phenotype in the untreated *ndr1-1* plants was caused by an internal accumulation of bioactive GAs in conjunction with enhanced sensitivity to GA signaling, we measured the *in planta* levels of GA_3 and GA_4, both of which are bioactive forms of GA that promote flowering in *Arabidopsis*[33,36]. The levels of GA in the mutant and WT were quantified under various growth conditions and at various stages of growth using HPLC and GC/MS-MS. *In planta* concentrations of the active GAs, GA_3 and GA_4, were significantly higher in *ndr1-1* plants by the third week of growth relative to the WT Col-0 plants (Fig. 3c).

Exogenous application of GA further enhances the floral transition in *ndr1-1* plants

We further explored the role of GA in the induction of early flowering in *ndr1-1* plants. Our first approach was to test whether the *ndr1-1* plants still retained responsiveness to external application of GA, which is known to accelerate flowering, or if the GA response was already saturated due to the enhanced levels of GA *in planta* due to feedback regulation[33,34].

Application of exogenous GA to *ndr1-1* plants reduced the time to floral transition. Though a reduction in time to flowering in response to GA was observed in the *ndr1-1* mutants, the effect of external application of GA towards the acceleration in bolting and flowering time was more pronounced in WT Col-0 plants around the fourth week by which time the *ndr1-1* plants began to show slower incremental growth (Fig. 4a, b, d and e). Exogenous application of GA significantly accelerated the flowering time in the GA treated *ndr1-1* plants compared with the untreated *ndr1-1* plants (Fig. 4a, top panel) and (Fig. 4b and c), though this difference was for shorter duration compared to the effect of GA treatment in WT Col-0 plants. This suggests that the *ndr1-1* plants retain responsiveness to exogenously applied GA at early stages of development. Exogenous application of GA significantly accelerated flowering in *ndr1-1* plants, but this phenotypic difference was short-lived relative to the response of the WT Col-0 plants (Fig. 5b) due to rapid growth of plants in the long-day growth conditions as well as the effect of GA application.

ndr1-1 plants display enhanced paclobutrazol resistance

Since early flowering and accelerated development in *ndr1-1* plants was associated with higher GA levels *in planta*, we tested whether this phenotype could be abolished by application of an inhibitor of GA biosynthesis. A single treatment of the GA biosynthesis inhibitor Paclobutrazol (PAC)[32,35], was applied to the base of both WT Col-0 and *ndr1-1* plants during the second week of growth, and flowering time and rosette size were measured in both genotypes.

The application of PAC lengthened the time to flowering in both the WT Col-0 and *ndr1-1* plants, indicating that GA biosynthesis was being effectively blocked in both genotypes (Fig. 5a, b and c). Furthermore, the rosette sizes in both genotypes were similar to those observed following PAC treatment, but bud initiation was much more rapid in the untreated *ndr1-1* mutant compared to either the untreated control WT Col-0 or the PAC-treated *ndr1-1* plants (Fig. 5a, top panel/enlarged inset and Fig. 5b), indicating that initiation of the visible bud, rapid bolting in terms of increased main bolt length and early opening of the terminal flowering phenotypes in the untreated *ndr1-1* plants were most likely due to an initial higher GA content *in planta* and an enhanced GA response (which though is slower than untreated plants) that was still functional at an elevated level in the mutant *ndr1-1* compared to the WT Col-0 despite the growth inhibition imposed by PAC. This was again apparent from the observation that the mutant *ndr1-1* plants though stunted as a result of the PAC treatment, still displayed the accelerated development parameters including early flowering when compared with WT Col-0 plants (Fig. 5a, bottom panel) as well as increased main bolt length and total silique number with respect to PAC treated WT Col-0 plants (Fig. 5d and e).

External GA application rescues the PAC associated phenotype of the *ndr1-1* plants

To test the possibility that the *ndr1-1* plants not only had higher levels of intrinsic GA, but also retained an enhanced response to the hormone, PAC-treated or untreated plants were subjected to a regimen of exogenous foliar GA_3 application with water-treated plants serving as control treatment for the overall experiment. Plants were monitored for the accelerated development and early flowering. With repeated application of GA, the phenotypic differences initially observed in response to PAC between the untreated plants of both genotypes including rescue of the delay in

flowering and reduction of bolt length were reversed. But strikingly, the PAC + GA-treated *ndr1-1* plants retained a slight but significant acceleration in flowering time as well as marginal increase in bolt length compared with the *ndr1-1* untreated plants (Fig. 6a, b and c). This subtle difference indicates an enhancement in GA signaling in the *ndr1-1* plants with repeated application of external GA while in the presence of a GA biosynthetic inhibitor (Fig. 6b, c and d). This also implies that *ndr1-1* mutants not only have a higher level of bioactive GA *in planta* but also retain an enhanced GA response, with the latter phenotype more likely having a greater contribution towards the various phenotypes observed over the entire developmental period of plants.

Genome sequence analysis of the *ndr1-1* mutant

The genome of the *ndr1-1* mutant used in this study was sequenced revealing that the 5' UTR and a portion of the coding region of *ndr1-1* were deleted (Supplementary Figure 3). There was no other detectable large insertion/deletion in our analysis, but rather only SNP differences between Col-0 and *ndr1-1* samples attributable to multiple generations of self-fertilization. In further support that there was no second site mutation that influenced the *ndr1-1* mutant phenotype, hundreds of *ndr1-1* plants examined in these experiments did not reveal segregation for pathogen susceptibility or early flowering phenotype.

Discussion

In addition to the role of the *Arabidopsis NDR1* in SA-mediated plant defense responses, the findings presented herein demonstrate a role for *NDR1* in the regulation of GA-mediated development, including flowering. The early flowering phenotype observed in the *ndr1-1* plants was accompanied by an increase in the expression of the regulatory genes that control the transition

to flowering and GA metabolic genes. Importantly, the whole genome sequence analyses of the *ndr1-1* mutant revealed no major deletion or insertion mutations in the sequence, and there was no segregation observed for the susceptibility or early flowering phenotypes of the *ndr1-1* mutant, suggesting a high improbability that a second site mutation was responsible for these stable phenotypes observed. Based on these results, we hypothesize that enhanced GA biosynthesis and signaling in *ndr1-1* plants results in an early flowering phenotype.

Enhanced GA production and signaling in *ndr1-1* plants opens an exciting framework to study the hormonal cross-talk between defense-related SA and other growth-regulating hormones under normal growth and under abiotic and biotic stress. Our discovery of *ndr1-1* as an early flowering mutant implicates *NDR1* as a negative regulator of flowering in *Arabidopsi*s under untreated conditions. The transition to flowering is further slightly but significantly accelerated in *Verticillium*-challenged *ndr1-1* plants as opposed to the untreated *ndr1-1* plants, indicating that *NDR1* may additionally play a negative role during induction of flowering by pathogen infection in the otherwise healthy plants.

We hypothesize that NDR1 acts as a major regulator of the balance between defense and development while helping to maintain cellular integrity, as a plasma membrane-localized integrin type protein[9,10]. The role of *NDR1* in enhancing plant defense responses to pathogen challenge may come at the cost of slower growth, delayed development, and an extended vegetative state due to delayed/suppressed transition to flowering. Tradeoffs in resource usage between nutrition and defense in plants have been well characterized[37], and hormonal imbalances during pathogen infection have important implications for plant productivity[38].

Switching cellular programs from an extended vegetative state to a defense-primed and accelerated reproductive state in the event of pathogen attack is an important consideration given

the sessile lifestyle of plants. We posit that such a strategy would, in theory, lead to a "relief of repression" on growth, resulting in transition to an accelerated reproductive state during pest/pathogen attack. This ultimately would result in enabling the plant to propagate seeds and "escape" to the next generation. Based on our observation that *ndr1-1* mutants have increased concentrations of active GA relative to WT Col-0, as well as infected *ndr1-1* plants flowering faster than uninfected *ndr1-1* plants, we hypothesize a potential role for GA in relief of repression of floral transition during the priming of plant defense responses to *Verticillium* spp. Using these observations as a foundation for further work in this area, we propose to explore the implication(s) for cross-talk between SA, the classical defense-related hormone, additional plant hormones (e.g., JA, ethylene), and the growth-regulating hormone GA, as summarized in Figure 7.

Historically, plant breeding for major disease resistance genes, including *NDR1,* comes at the cost of altered flowering response and overall productivity, phenotypes that could effectively negate the benefits of fortified resistance to pathogens. Apart from assigning new roles to a key regulator of defense signaling in plants, our findings also shed light on a new mechanism associated with hormone regulation of one of the major defense regulators in plants. This latter finding reinforces the fact that precaution must be taken to understand gene functions and regulatory activities of any gene product before targeting them as candidates for crop improvement.

Methods

Plant growth conditions

Arabidopsis thaliana seeds were surface-disinfected with 10% (v/v) commercial bleach solution with a few drops of 10% SDS (Sigma) and subsequently washed at least thrice and

incubated at 4°C in sterile water in the dark for 3-7 days to break dormancy. Sterilized and cold-treated seeds were mixed in 0.01% top agar (Difco Agar, Beckton, Dickinson and Company, MD, USA) and were directly sown into Sunshine Plant Growth Soil Mix No.1 in trays. The plants were grown under either short day (S/D) photoperiod (10/14 h) or, long day (L/D) photoperiod (14/10 h)- day/night (D/N) cycle with a 22°C/20°C D/N temperature cycle in climate controlled growth chambers (Conviron, Winnipeg, Canada). Lighting was provided by cool white light emitting bars at a fluence rate of 130-150 µmol photons s^{-1} m^{-2} under both light regimes. Some experiments were repeated under greenhouse conditions where plants received natural light supplemented with overhead mercury lamps at night to maintain 16/8 h D/N cycle as required. Greenhouse temperature was maintained at 22 $\pm$2 °C for all experiments. All plants were fertilized once every two weeks (Jack's Classic, J. R. Peters, Inc., PA), starting from the second week of growth until the completion of senescence. For bolt measurements, plants were grown in growth chambers for at least eight weeks, then moved to greenhouse. Intact plants were allowed to dry, and final measurement were taken soon thereafter.

Pathogen culture and plant treatment

Two species of vascular wilt pathogens were used in this study. *Verticillium dahliae* from lettuce has a broad host range and the host range of *Verticillium longisporium* is restricted to plants in the brassicaceae[6,19,20,26,39]. Lettuce isolates of *V. dahliae* used in this study included VdLs.16 (California isolate, 1996) and VdLs.17 (ATCC accession MYA-4575) which represent races 1 and 2, respectively. The isolate of *V. longisporum* from cauliflower used was VlBob.70 (CA, 1990)[40]. All *Verticillium* isolates used in the study were grown at room temperature on potato dextrose agar (PDA) (Difco, Beckton, Dickinson and Company, Maryland, USA) supplemented with

cholarmphenicol (0.05g/l) (Fisher Scientific) and Streptomycin Sulfate (0.05g/l) (Mediatech, Inc., Herndon, Virginia) from a 20% glycerol stock culture stored at -80°C. After a week of growth, fresh agar plugs with visible mycelia were transferred on to freshly prepared PDA plates that were grown at room temperature for 2 weeks before use. Pathogen treatments consisted of *Verticillium* conidia suspensions prepared by flooding the 2-week-old cultures on PDA with sterile distilled water and conidial counts were determined with a hemocytometer and a compound microscope (Olympus BX60, Japan) and adjusted to a concentration of $1x10^7$ conidia/ml in distilled water. Conidial suspensions were stirred to prevent settling of conidia and 5 ml of the conidia suspension was dispensed into the potting mix near the crown of the plant during the second week of plant growth. Whenever the experiment was carried out under greenhouse conditions, natural day light was augmented with mercury lamps to maintain long day conditions with a 16/8 h D/N cycle.

For gene expression analysis in plant tissues that were inoculated with a pathogen, samples were collected at the indicated days post inoculation (dpi) and immediately placed in liquid nitrogen and stored in a -80°C freezer until further use. All experiments were carried out at least two times with similar results in climate controlled growth chambers and greenhouses, unless mentioned otherwise.

Plant flowering time and growth measurement

Arabidopsis flowering time was measured by scoring the plants with open terminal flowers (TFO), as described previously[24]. The number of rosette leaves (RLN) were counted after all the plants in both the genotypes flowered. Around 25 plants of both WT Col-0 and *ndr1-1* were used throughout the study for each experimental replicate unless indicated otherwise. Main bolt length (MBL) was measured beginning on the day of plant flowering. Subsequently, the process was

repeated every week for all the bolting plants in a population until all of the plants flowered, ceased visible growth, and started to senesce. Plants were moved to a greenhouse to dry out completely and the final tallies of MBL, silique per main bolt (SMB), and total silique number (TSN) were recorded for all the plants in both genotypes. The average MBL over the duration of growth and average of the number of SMB was used as a quantitative indicator of growth, beginning at the time of bolt initiation, and during the subsequent days until visible growth ceased. Genotypes susceptible to *Verticillium* spp. showed stunted bolts and reduced rosette size among other disease features, as previously described in other plant-pathogen systems[6,26].

For analysis of the expression of the genes regulating flowering time, 7-to-9-day-old soil sown seedlings were grown in climate controlled growth chambers under long day conditions of a 14/10 h D/N cycle and were harvested for analysis at the end of the light period. At least 50-70 seedling samples of the respective plant genotypes were collected for the gene expression analyses. All of the experiments and measurement criteria were replicated at least three times with similar results.

Chemical treatments

Bioactive GA_3 (Sigma-Aldrich) or Paclobutrazol (PAC; Alfa Aesar) were used at final working concentrations of either 50 μM or 100 μM for GA_3 in tap water containing 0.015% Silwett-S77 (Lehle seeds, Texas, USA) and 35mg/l of PAC in tap water. The GA_3 solution was applied as a fine spray (with a store brought spray bottle) until the plants dripped with the sprayed solution. Paclobutrazol was applied as a solution directly to the soil in the pot containing the seedlings of each genotype at a rate of 5ml/pot for all the experiments, beginning at the second week of growth, unless otherwise noted.

When GA_3 and PAC treatments were administered simultaneously, PAC was first applied to the soil followed immediately with a foliar spray of GA_3. One application of PAC was enough to induce the difference between the *ndr1-1* and the wild genotypes, while GA_3 sprays were administered twice within one week till the plants ceased any visible growth and distinctly displayed senescence.

Statistical analysis of phenotype data

The Welch's *t*-test was used as a two-sided test for comparison of mean using the R statistical software (R Core Team 2017) [45]. A *P*-value of < 0.05 was considered to be statistically significant.

Gene expression analyses

Arabidopsis seedlings or leaf samples were collected in 1.5 ml centrifuge tubes and flash frozen in liquid nitrogen at indicated time points. Samples were collected from 50-70 individual plants per treatment for each time point and used for gene expression analysis. Total plant RNA was isolated using Trizol reagent (Invitrogen) and quantified using a Nanodrop spectrophotometer (ThermoFisher). Total RNA (10 μg) was reverse transcribed into cDNA using SuperScriptIII reverse transcriptase (Invitrogen) for reverse transcription quantitative PCR (RT-qPCR) analyses. The RT-qPCR assays were performed on a LightCycler480 II (Roche, Germany) using ABI TaqMan Gene Expression Master Mix (ThermoFisher). The relative expression of each gene of interest was calculated using the REST software (Qiagen). The TaqMan probes used were obtained from ThermoFisher, and the assay identification numbers for assays are attached in supplementary information section.

391 **Plant genotypes and mutant information**

392 Both the *ndr1-1* and WT Col-0 seeds were initially obtained from the Arabidopsis Biological
393 Resource Center (ABRC; Columbus, Ohio, USA). The homozygous *ndr1-1* mutant is in the Col-
394 0 background and is a loss of function mutant. To synchronize the seed quality and seed age, seeds
395 of *ndr1-1* and WT Col-0 were also obtained by allowing the soil grown plants to self-fertilize and
396 senesce, followed by seed drying on the plants for at least 40-60 days. Both genotypes were grown
397 on the same tray under similar growth conditions throughout. Seeds were collected from at least
398 two generations of plants as described above. For the experiments described above, similar results
399 were obtained using these seeds in multiple replicates. The mutant genotype was confirmed each
400 time by qPCR and by sequencing for the absence of deleted region in the *ndr1-1* mutant (citation).

401 **DNA Extraction and Whole Genome Sequencing**

402 DNA was extracted from four-week old Arabidopsis plants as described[41] and quantified. The
403 whole genome of the *ndr1-1* mutant genotype was sequenced by Illumina MeSeq platform with
404 paired end 150nt mode in the Genomics Core Facility at Michigan State University (xxx, MI).
405 Raw DNA sequencing reads were filtered and trimmed using Trimmomatics v0.33[42] and mapped
406 to Arabidopsis reference genome TAIR10 using bowtie2 v2.2.6[43]. For visualization purposes
407 alignments of ndr1-1 and Col-0 genome were visualized in the genome viewer Tablet[44]
408 (Supplementary Figure 3). Raw read sequence of *ndr1-1* is deposited to NCBI short read archive
409 (SRA) under BioProject PRJNA489296.

410

411 **Measurements of gibberellic acid**

412 GA_3 was measured by Shimadzu Liquid Chromatograph 2030-C model coupled with a UV detector
413 at 208 nm. It consists of a multi-solvent delivery system, a water in-line Degasser AF and water

414 temperature control system. Shimadzu C18 column (2.7 µm particle size, 150 mm x 4.6 mm i.d.) was used
415 at a flow rate of 1.0 ml min-1. The sample injection volume was 20 µL and oven temperature was
416 maintained at 20ºC. The mobile phase was acetonitrile-water at a ratio of 25:75 (v/v). GA_3 concentration
417 was calculated from external standard curves.

418 GA_4 and GA_9 were measured by using Shimadzu LC-MS/MS 8050 equipped with C18 reversed phase
419 column (3 µm particle size, 50 mm x 4.6 mm i.d.) by using acetonitrile-water at ratio of 5:95 (v/v) as the
420 mobile phase. GA_4 and GA_9 were ionized by Electro Spray Ionization mode with heat block temperature
421 of 400ºC and interface temperatures of 300ºC. The DL temperature was 300º C and the interface voltage
422 was +3.5 kV. LC MS/MS confirmation transition conditions for GA4 and GA_9 were 331>287 and
423 315>253, respectively. Hormone quantification was performed using external standard curves for GA_4
424 and GA_9 from plant total extracts.

425

426

427 **Additional Information**

428 **Competing Interests:** We declare that the authors have no competing interests as defined by
429 Nature Research, or other interests that might be perceived to influence the results and/or
430 discussion reported in this paper.

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Author Contributions

N.D., S.J.K., D.P.G.S., and K.V.S. wrote the main manuscript text; N.D., S.J.K., and K.V.S. conceived experiments; N.D. and D.P.G.S. conducted experiments; A.A. performed RT-qPCR analyses of gene expression; H.A. performed mass spectrometry analysis; N.D. prepared all figures. All authors approved the final version of the manuscript.

Figure legends

Figure 1: ***Arabidopsis ndr1-1* mutants are susceptible to *Verticillium* and display accelerated flowering upon infection.** Phenotype of the *V. longisporum* (VlBob.70)-treated WT Col-0 (left) with respect to the susceptible *ndr1-1* mutant (right) shown at three weeks post inoculation **(a).** Average time to flower in terms of rosette leaf number (RLN) **(b),** average time to flower in terms of appearance of the visible bud (VB) **(c)** and the appearance of open terminal flower (TFO) **(d)** on the main axis is shown as days post germination (dpg) for the genotypes treated with *Verticillium dahliae* (*Vd*Ls.16 and *Vd*Ls.17) and *Vl*Bob.70 (*Vl*) and water treated control (mock), for both WT Col-0 and *ndr1-1*. Average final main bolt length (MBL) for WT Col-0 and *ndr1-1* treated with the above pathogens after the plants ceased any visible growth **(e)**. Average number of siliques on the main branch (SMB) for WT Col-0 and *ndr1-1* treated with the above pathogens after the plants ceased any visible growth **(f)**. Figures shown above are representative of data from one experiment. The experiments were repeated at least twice with similar results with 20-25 plants per genotype. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$ (two-sided Welch's t-test). Bar, SD.

573 **Figure 2**: **Early flowering and accelerated development of the Arabidopsis *ndr1-1* mutant.**
574 The early flowering phenotype of *ndr1-1* plants (left) with respect to Columbia (Col, right) at four
575 weeks (**a**), six weeks (**b**) and eight weeks (**c**) of growth. The *ndr1-1* plants flower early and bolt
576 faster (**a**) but the growth is slowed eventually with respect to WT Col-0 (**b**) and is eventually
577 overtaken by the WT Col plants (**c**). Average time to flower in terms of appearance of the visible
578 bud (VB) (**d**) and when the first terminal flower opened (TFO) **(e)** on the main axis is shown as
579 days post germination (dpg) for genotypes shown in the figure. Average number of leaves/plant
580 (both rosette leaf number and cauline leaf number) is as shown **(f)**. Average final Main bolt length
581 (MBL) at the time of flowering is shown in (**g).** Main bolt length (MBL) at the time of opening of
582 the terminal flower is shown in **(h)**. Average MBL at the indicated time points for both the
583 genotypes until plant senescence and cessation of growth **(i)**. Average number of siliques per main
584 bolt (SMB) at the cessation of growth (**j)** and the total number of siliques per plant (TSN) is shown
585 in (**k)**. Figures shown above are representative of data from one experiment. Approximately 20-
586 25 plants per genotype were used for the measurements and the experiment was repeated at least
587 twice with similar results. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$ (two-sided
588 Welch's t-test). Bar, SD.
589
590 **Figure 3**: **Analyses of flowering time and gibberellic acid biosynthetic gene expression and**
591 **gibberellic acid levels in *ndr1-1* Arabidopsis plants.** Reverse transcription-quantitative PCR
592 (RT-qPCR) analyses of gene expression were performed for genes controlling the transition to
593 flowering including the positive regulators *CONSTANS (CO)*, *FLOWERING LOCUS T (T)*,
594 *SUPPRESSOR OF CONSTANS 1 (SOC1)*, *LEAFY (LFY)* and *APETALA 1 (AP1)***.** The expression
595 was normalized with respect to *UBIQUITIN 10 (UBC10)* gene **(a)**. At least 50-70 seedlings per

596 genotype from soil grown plants under long day conditions were used and the experiments were
597 replicated at least twice with similar results. Error bars represent the standard error between these
598 replicates for each gene tested. RT-qPCR was also performed on selected genes involved in
599 biosynthesis of the bioactive GAs including *GA3ox* and *GA20ox* (right panel), and genes involved
600 in the deactivation of bioactive GA by 2-β hydroxylation including *GA2ox6* and *GA2ox7* (left
601 panel) **(b)**. Bioactive GA levels *in planta* (**c**). Aerial parts of 20-30 2 to 3-week-old, long-day
602 grown plants were harvested mid-day in liquid nitrogen, and were processed as described in the
603 methods. Bioactive forms of GA, GA_3 and GA_4, were measured by HPLC and GC-MS using
604 known standards and as described in methods. The error bars represent the standard error of the
605 average of two independent biological replicates.
606
607 **Figure 4**: ***ndr1-1* mutants retain enhanced gibberellic acid responses.** Col and *ndr1-1* plants
608 were sprayed with 100 μM GA_3 dissolved in water containing 0.015% Silwett-77 (top panel) and
609 with just water containing 0.015% Silwett-77 (bottom panel) for control treatment (mock) **(a)**.
610 Average time to flower in terms of the terminal flower open (TFO) and when the first terminal
611 flower opened (TFO) on the main axis is shown as days post germination (dpg) for the genotypes
612 shown in the figure **(b)**. Average rosette leaf number (RLN) is shown in (**c)**, while average main
613 bolt length (MBL) and average silique number on the main bolt (SMB) at 37 dpg is shown in **(d)**
614 and **(e)** respectively. Figures shown above are representative of data from one experiment.
615 Approximately 20-25 plants per genotype were used for the measurements and the experiment was
616 repeated at least twice with similar results. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$
617 (two-sided Welch's t-test). Bar, SD.
618

Figure 5: *ndr1-1* plants display enhanced paclobutrazol (PAC) resistance. The gibberellic acid (GA) biosynthesis inhibitor paclobutrazol (PAC) was applied as a 35 mg/l solution in water. Top panel shows the visible difference in emergence of the floral bud as it appears to the naked eye from representative plant samples treated with PAC (**a)**. Average time to flower in terms of appearance of visible buds (VB) **(b)**. Average total leaf number [both rosette (RL) and cauline leaves (CL)] is shown in **(c),** while the average main bolt length at flowering at the cessation of any visible growth and the total silique number from the whole plant is shown in **(d)** and **(e)** respectively. Figures shown above are representative of data from one experiment. Approximately 20-25 plants per genotype were used for the measurements and the experiment was repeated at least twice with similar results. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$ (two-sided Welch's t-test). Bar, SD.

Figure 6: Hormonal (GA) rescue of PAC mediated growth inhibition confirms enhanced GA reception as the basis of early flowering phenotype of *ndr1-1* plants. The gibberellic acid response inhibitor (PAC) was applied as a 35mg/l solution in water directly to the pots (middle and right panels**) (a)**. For GA treatment, plants shown in the same panels were treated twice per week with either 100 μM GA_3 dissolved in water containing 0.015% Silwett-77 (middle and right panel) or with water containing 0.015% Silwett-77 (left panel) as a control treatment. Flowering time is shown as the average time to flower for the terminal flower to open (TFO) for WT Col-0 and *ndr1-1* treated with either GA, or in combination with PAC, along with water treated plants as a control (mock) is shown in **(b)** while the total rosette leaf number (RLN) after all the plants flowered is shown in **(c)**. Main bolt length (MBL) was monitored weekly at indicated days post-

642 germination (dpg) for comparing the growth between WT Col and *ndr1-1* plants subjected to the
643 GA and PAC treatments (**d)**. Figures shown above are representative of data from one experiment.
644 Approximately 20-25 plants per genotype were used for the measurements and the experiment was
645 repeated at least twice with similar results. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$
646 (two-sided Welch's t-test). Bar, SD.
647
648 **Figure 7**: **Model depicting the effect of the *ndr1-1* mutation on physiological processes**
649 **relative to the previously defined role of *NDR1*.** A *loss-of-function* (*ndr1-1*) mutation in *NDR1*
650 diminishes/abrogates the hypersensitive response (HR) and systemic acquired resistance (SAR)
651 known to involve Salicylic Acid (SA) pathway, and also reduces the accumulation of SA in
652 response to pathogens. In this study, we demonstrate that the levels of the phytohormone GA are
653 elevated in untreated *ndr1-1* plants, a phenomenon we hypothesize contributes to the early
654 flowering phenotype. We also demonstrate that *ndr1-1* plants have accelerated growth and display
655 early senescence as a result of the *NDR1* deletion. Dotted lines represent findings from this current
656 study, while the solid lines represent established results from multiple research groups since the
657 discovery of the effect of *ndr1-1* on defense responses in *Arabidopsis*.
658
659 **Supplementary Figure S1**: **Early flowering of *Arabidopsis ndr1-1* plants under short day**
660 **conditions.**
661 Early flowering phenotype of *ndr1-1* plants (right) in comparison to the WT Col (left) at seven
662 weeks of growth. The early flowering phenotype was observed in more than three replicates with
663 at least 15-25 plants per genotype. Growth differences under short day conditions (10/14 h

light/dark cycle) with *ndr1-1* plants flowering early and bolted faster with respect to WT Col-0 plants, similar to the differences observed under long day conditions (14/10 h light/dark cycle).

Supplementary Figure S2: ***Arabidopsis* plants with *ndr1-1* mutation have reduced seed yield.**

Average total seed weight in *ndr1-1* compared to WT Col-0 plants, represented as grams per similar number of plants tested (**a**). This phenotype was observed in multiple replicates under our growth conditions with at least 15-25 plants per genotype. Total seed weight was determined in grams for the same number of plants of either WT Col-0 or *ndr1-1* plants (**b**). Similar results were also obtained with plants grown under long day conditions in both climate controlled growth chambers and green house.

Supplementary Figure S3: Illumina genome sequencing of the *ndr1-1* mutant.

Sequencing of genomic DNA (gDNA) from 4-week-old leaves from the *ndr1-1* mutant reveals a deletion of 1,233 base pairs (bp), encompassing 596 bp deletion, 5' of the *NDR1* UTR. The wild-type Col-0 genome sequence was used as a reference for mapping reads from the *ndr1-1* mutant. Read depth is illustrated based on the linear sequence (i.e., rows), with colors denoting individual nucleotides (green="A", purple="T", red="G", and orange="C"). Nucleotide positions on chromosome 3 are shown as points of reference for the location of the *NDR1* deletion.

Supplementary Table 1: **List of all the TaqMan assay ID numbers used for reverse transcription-quantitative PCR analysis in this study.**

Gene Name	ATG Number	TaqMan Assay ID
GI	AT1G22770	At02305659_g1
CO	AT5G15840	At02200179_g1
FT	AT1G65480	At02224075_g1
SOC1	AT2G45660	At02263351_m1
LFY	AT5G61850	At02270390_m1
AP1	AT1G69120	At02226237_g1
NDR1	AT3G20600	At02262314_s1
GA3 OX1	AT1G15550	At02155986_g1
GA3 OX2	AT1G80340	At02289801_g1
GA20 OX1	AT4G25420	At02238367_g1
GA20 OX2	AT5G51810	At02320185_g1
GA2 OX6	AT1G78440	At02259101_m1
GA2 OX7	AT1G30040	At02272024_m1
UBC10	AT5G533000	At02234192_gH

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