1 **Phytohormonal cross-talk modulate *Bipolaris sorokiniana* (Sec.)interaction with Zea**
2 **mays**

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15

16 Abstract

17 Besides acting as growth inducing molecule, Gibberellin (GA₃) also confers the compatibility
 18 of microbial interactions with host. We inoculated 11 days old *Z. mays* seedlings grown under
 19 hydroponic conditions and high GA₃ levels with *Bipolaris sorokiniana* (BIPOL) at the spore
 20 density (SD) of OD_{0.6}. The high level of GA₃ negatively affected the growth of the seedlings,
 21 accompanied by the high level of stress deducing secondary metabolites (proline, total
 22 flavanoids, phenylpropanoids, and glucosinolides). Moreover, high level of GA₃ produced a
 23 hypersensitive response (HR) in the seedlings. The HR developed cross talks with IAA and
 24 trans-zeatins and triggered higher production of hypersensitive inducing biomolecules. The
 25 other HR co-related biological processes were demonstrated by high phytoalexins level and
 26 high protease activities. Such activities ultimately inhibited the colonization of BIPOL on the
 27 roots of maize seedlings. The products of the genes expressed at high GA₃ also conferred the
 28 deterrence of BIPOL colonization at SD = OD_{0.6}. Intriguingly, when we inhibited GA₃
 29 biosynthesis in the seedlings with aerielly sprayed uniconazole, prior to BIPOL treatment, the
 30 BIPOL colonized and subsequently promoted the seedling growth. This low level of GA₃
 31 after BIPOL treatment checked the high level of secondary metabolites and hypersensitivity
 32 inducing molecules. The results, thus suggested that the aforementioned processes only
 33 happened in the BIPOL at SD (OD_{0.6}), whereas the SD at lower levels (OD_{0.2} or OD_{0.4})
 34 neither promoted the growth of uniconazole pre-treated seedlings nor produced HR in control
 35 seedlings of maize plant.

36 **Keywords:** Plant-micorbes interaction, hormonal cross talks, growth activity, Uniconazole,
 37 *Zea mays*

38

39 **Introduction**

40 Upon the plant microbe interaction, several hypersensitive reactions, including hormonal
41 biosyntheses and the subsequent signal transduction mechanism are triggered (Kim et al.,
42 2018). Such signal transduction in host later develops the expression of genes which decide
43 the fate of microbe interaction with host (Ramirez-Prado et al., 2018). Recently, gibberellins
44 hypersensitivity accompanied by their signal transduction processes have emerged as a
45 critical component of plant-microbe interactions (Binenbaum et al., 2018). However, very
46 little information is available about the cross talk between GAs and other phytohormones in
47 plant under stress conditions (Zhang et al., 2018). On the other hand, gibberellins signalling
48 are widely manipulated by the proteolytic activity of DELLA proteins (Xiang et al., 2018).
49 Most of the interacting plant microbes suppress the proteolysis of DELLA proteins in host
50 plants by secreting a variety of compounds (Brumos et al., 2018).

51 Hypersensitive response (HR) in plant is triggered upon the interaction of undesired microbe
52 for host plant (Matić et al., 2016). HR in plant is characterized by the production
53 hypersensitive inducing molecules such as c-di-GMP, and cAMP (Cadby et al., 2019). Other
54 signalling molecules such as phosphatidic acid (PA), free or esterified oxo-phytodeinoic acid
55 (OPDA), and jasmonic acid (JA) are also at the site of HR in host (Hou et al., 2016). These
56 molecules activates the host defence response against the microbe (Lim et al., 2017).
57 Accompanied by this, the host plant produces phytoalexins (Suárez et al., 2018) and high
58 protease activity (Asai and Shirasu, 2015). Primarily, such HR is activated at the response of
59 high level of phyto-hormone (Großkinsky et al., 2016). In plant, IAA, GA₃ and trans-zeatin
60 are responsible for the HR (Body et al., 2019).

61 During HR to any stress, the host plant growth is adversely affected which is optimally be
62 determined by Relative Growth Rate (RGR) (Vasseur et al., 2018) and Net Assimilation Rate
63 (NAR) (Koch et al., 2019) at different time duration of treatment. In such condition, plant

64 increases the concentration of secondary metabolites in its leaf and roots which combat any
65 stressing condition (Yang et al., 2018). The commonly determined secondary metabolites in
66 the plants are proline (Silva et al., 2018), flavonoid contents (Tohge et al., 2018), phenyl
67 propanoids (PPs) (Hiruma, 2019), and glucosinolates (GLs) (Czerniawski and Bednarek,
68 2018). Phyto-hormonically in host, HR inducing microbes suppress the E3 ligase
69 polyubiquitination to inhibit GA₃ from degrading DELLA protein (Li et al., 2019). Most of
70 the arbuscular fungi (AF) are known to utilize GA₃ signalling in order to produce nodulation in
71 plant (Mamontova et al., 2019). Pea mutant *cry-s* is known to have high AF colonization due
72 to reduce level of GA₃ and high DELLA protein activity (McGuinness et al., 2019). Fungal
73 sporulation specifically needs low GA signalling in host as an optimal environment (Bedini et
74 al., 2018). The plant host usually discourages the biotroph growth in their tissues by releasing
75 GA₃ and associated signalling with it (Yimer et al., 2018). It is also worth mentioning that the
76 surface elicitors of the microbes also contribute to gibberellins hypersensitivity (Mott et al.,
77 2018).

78 Surface elicitors that are responsible for gibberellins hypersensitivity in host, includes
79 glycoproteins and Glycolipid (Chaliha et al., 2018). Besides, high level of GA₃ in host plants
80 also interferes with the other phytohormones (Tijero et al., 2019). GA₃ is known to develop
81 antagonistic relation with other plant growth hormones, once its level significantly increases
82 beyond the optimum requirements in the host plants (Fuentes et al., 2019). The production of
83 higher amounts of GA₃ thus interferes with the growth and development of the host plants
84 through establishing of cross talks between GA₃ and other phytohormones (Feurtado and
85 Kermode, 2018).

86 *Bipolaris* is genus of higher fungi frequently found in the plant debris and soil.
87 (Kurosawa et al., 2018). Although, various species of *Bipolaris* are reported to have growth
88 promoting agent in plants (Nandhini et al., 2018) but there are some other species which are

pathogenic in telomorphic stage, such as *Bipolaris hawaiiensis*, *Bipolaris spicifera*, and *Bipolaris australiensis* (Nur Ain Izzati et al., 2019). *Bipolaris sorokiniana* is one of the dubious species of *Bipolaris* which acted as hemibiotroph and cause HR in variety of plant species (McDonald et al., 2018). Similarly, at several places, this fungus have been reported as endophytes (Khan et al., 2015). Therefore, there is a wide gap of research to describe the mode of infection of *B. sorokiniana* (BIPOL). Previous studies suggest that the manipulating role of the host GA₃, IAA and TZn is highly unknown during the interaction of BIPOL to host plant.

Presently, we have analysed the GA₃ hypersensitivity at the interaction of BIPOL with spore densities (Tohge et al.) OD_{0.2} OD_{0.4} or OD_{0.6} and its cross talks with IAA and cytokinins. Also, it was determined that how GA₃ cross talks with IAA and trans-zeatin can effect BIPOL colonization in host root (maize seedlings) and the related host growth responses.

MATERIALS AND METHODS

Plant materials

Seeds of *Zea mays* (var. *Hysun-33*) were obtained from BCSS (Bio Care Service Seeds) and kept at 4 °C for 21 days for vernalisation period (Müller et al., 2017). At the time of experiment, the seeds were treated with dilute solution of HgCl₂ (0.1%) for 10 seconds and then surface sterilized with 70 % ethanol (Sen et al., 2013). The sterilized seeds were washed with autoclaved distilled water to rinse off any surface adsorbent. The sterilized seeds were then shifted to Petri plate having filter paper moistened with distilled autoclaved water. Afterwards, the Petri plates were wrapped in an aluminium foil and incubated at 25 °C for 3 days. Germinated seedlings of same vigour were selected and shifted to pots having standardized Hoagland solution as described (Hassan, 2017). The seedlings were acclimatized in the Hoagland solution for 3 days and then the seedlings were shifted to four

different sets to receive different treatment. The first set of maize seedlings were treated with 2 mL fungal spore in Hoagland solution to grow (B). Leaves of second set of maize seedlings were sprayed with 2 mL 10 mM yucasin (Y). The third set received both treatment as fungal inoculation at roots of the seedlings after 12 hours pre-treatment of Yucasin (Y-B). Control seedlings did not receive any of the above mentioned treatment (C).

Preparation of fungal inoculum for infection assay

The culture of previously isolated, identified and preserved BIPOL (Asaf et al., 2019) was refreshed at 28 °C on PDA. About, 0.02 g of the fungal spores were transferred to a 500 mL flask containing 250 mL of potato dextrose broth in the flask and then incubated at 25 °C for 5 days in a shaking incubator operated at 150 rpm (Khan et al., 2015). On the 5th day, 1 mL of the inoculum from the spore suspension was taken and transferred to a fresh potato dextrose broth. The fresh culture broth was kept overnight at 25 °C in a shaking incubator. Prior to inoculation, the spore was washed by centrifugating at 10⁵ xg at 10 °C for 30 min in sterile distilled water trice (Huttenlocher et al., 2019). Optical density (OD) of the washed spore's suspension was measured at 600 nm and adjusted to OD_{0.2} or OD_{0.4} or OD_{0.6} with the help of sterilized distilled water. We used germinated spore to inoculate the plant to accelerate the colonization potential of *B. sorokiniana* (Selvakumar et al., 2018).

Determination of fungal root colonization

Root segments of seedlings of the fungal treatment were kept on a Petri plate containing PDA media and then incubated at 25 °C. To calculate fungal colonization frequency, the relative number of the root segments occupied by the fungus was observed.

Preparation of Sample for biochemical analyses

For phytochemical analyses, fresh leaves were quickly frozen in liquid nitrogen and ground to obtain fine powder in a mortar and pestle. Absolute methanol (200 mL) was then added to the fine powder (2 gm) and the solution was transferred to the soxhlet apparatus for the

extraction of phytochemicals (Dhawan and Gupta, 2017). The obtained leaf extract (LE) was first filtered and the filtrate was concentrated in the rotary evaporatory (Wahyuningsih et al., 2017). Moreover, root exudates (RE) was obtained from filtered hydropenic culture of the root seedlings and concentrated in the rotary evaporatory.

Secondary metabolite determination

Colorimetric method was used for the evaluation of total flavonoids and proline contents. Samples (1 mL) of LE and RE were prepared as mentioned above, mixed in 3 % sulfo-salicylic acid (4 mL) and centrifuged for 5 mins at 6077 rcf. After centrifugation, the 2 mL of ninhydrin reagent was added to the samples and shaken vigorously. Acid ninhydrin reagent was prepared by adding 1.25 g of ninhydrin in pure glacial acetic acid (30 mL) and 20 mL of phosphoric acid (6 M) and mixed. The reaction mixture was heated for 1 hour at 100 °C and the pellet found was disappeared in toluene (4 mL) (Lee et al., 2018). OD of the samples were monitored using UV/Vis spectrophotometer (Lambda 1050) at 520 nm. Similarly, for determination of total flavonoid contents, 0.5 mL of LE and RE samples were added into 10 % potassium acetate (100 µL), 10 % aluminium chloride (100 µL), 70 % ethanol (4.3 mL). The mixture was incubated at room temperature for 30 minutes and the OD was measured at 450 nm on UV/Vis spectrophotometer (Lambda 1050) (Gil-Ramírez et al., 2016).

Phenylpropanoids and glucosinolates were determined using HPLC technique by taking ferulic acid (Genovese et al., 2018) and indolyl glucosinolates (Aghajanzadeh et al., 2019) as standards respectively. The samples (RE or LE) first were filtered through a 0.2 µm membrane filter associated in a syringe filtration. The filtrated sample (20µL) was then poured into a C18 reverse phase HPLC column and eluted with 75 % methanol (in % 10 acetic acid) for Phenylpropanoids and glucosinolides through an isocratic pump. The eluate was monitored through a UV detector set at 212 nm and 287 nm for Phenylpropanoids and glucosinolates respectively (Jeon et al., 2018).

Enzymatic activities determination

Oxidase and catalase activities were determined in the LE and RE samples (Röcker et al., 2016). For the determination of oxidase activity, sample (200 μ L) was mixed with 1.5 mL of phosphate buffer (50 mM), 200 μ L of ascorbic acid (0.5 mM), and 200 μ L of H₂O₂ (0.1 mM) in a cuvette. OD was measured with an interval of 30 seconds at 290 nm. The collected data was averaged for each sample and expressed in enzyme units per gram of tested sample (Ugm⁻¹). For calatase activity determination, reaction mixture was prepared by mixing 40 μ L sample with 400 μ L of H₂O₂ (15 mM) and 2.6 mL of phosphate buffer (50 mM). The OD was measured at 240 nm for each sample using the same procedure as for oxidase activity determination (18).

Similarly, Cofactor NAD⁺ (Nicotinamide adnenine dinucleotide) was determined by adding 2 mL sample (LE or RE) into 50 μ L MgCl₂ (500 mM), Tris-HCl Buffer (0.5 mL) and 100 μ L FADH. Reaction was started by adding 500 mL G6P (500 mM) into reaction mixture. OD was monitored on 340 through spectrophotometer (Hughes et al., 2015). Cofactor FAD⁺ (Flavin adnenine dinucleotide) was determined by adding 2 mL sample (LE or RE) into reaction mixture containing 1 mL HEPES (50 mM), 100 μ L ascorbic acid (250 mM) and 100 μ L FADH. Reaction was initiated by adding Metolachlor OA (C₁₅H₂₁NO₄). Optical density was monitored at 340 without any incubation through photo-spectrometer (Ceh-Pavia and Lu, 2016).

Intracellular determination of c-di-GMP and cAMP level

Freshly taken leaf or root section (2 gm) was homogenised by centrifugation at 5000 g, for 30 min. The cell pellet was suspended in 1 mL extraction buffer (40 % methanol, 0.1 % formic acid, 40 % acetonitrile, and 19.9 % distilled water) and vortexed for 30 sec. The sample was incubated for 30 min. after incubation, lysing was done in by using non-contact ultra-sonication (UCD-200, Belgium) (Jenal et al., 2017). The samples were subjected to HPLC-

MS/MS on QTRAP 4500 system (USA) (Jenal et al., 2017). The results was compared with the standards of the pure c-di-GMP and cAMP levels.

Quantification of hypersensitive inducing molecules

Quantification of hypersensitive inducing molecules as pure Oxo-phytodeinoic acid (OPDA), esterified OPDA, phosphatidic acid (PA) and jasmonic acid (JA). Root or leaf tissues (2 gm) were taken into glass tube containing 5 mL distilled water and agitated in orbital shaker. After removing root or leaf tissues, samples were acidified by adding 50 µL HCL (1.6 M). Phase separation was done through mixing ethyl acetate (2 mL) and then dried into nitrogen gas stream. The samples were then dissolved into methanol (50 µL) and subjected to HPLC-MS/MS on QTRAP 4500 system (USA). The results was compared with the standards of pure OPDA, esterified OPDA, JA and PA.

HPLC-ESI-MS/MS Analyses of phyto-hormones

Hormonal level including IAA (Indole-3-acetic acid), GA₃ (Gibberellin) and Trans-Zeatin (TZn) in the LE and RE was observed through HPLC-ESI-MS/MS. LE and RE (10 mL) centrifuged by 6744 rpm for 20 mins. The obtained supernatants were concentrated in vacuum centrifugation concentrator (Heto-Holten, Denmark) to 5 mL. The concentrated supernatant was first filtered through 0.22 mm disposable cellulose acetate membrane and then subsequent determination with by HPLC–ESI –MS/MS. For the analyses through HPLC–ESI –MS/MS, the aligent 1260 HPLC apparatus was connected with a aligent 6410B mass spectrometer equipped with Park nitrogen generator (11.0L/ min Nitrogen flow and a negative mode an electrospray ionization source (4000 V, 45 psi) and). Mobile phase was made by adding acetonitrile (0.1 % formic acid) into acidic water (0.1 % formic acid) in ratio of 2:1 at a flow rate of 0.5 mL/min for 40 min. Hormonal analysts (IAA, GA₃, TZn) were monitored at 312.9 m/z, 391.6 m/z, and 379.4 m/z respectively. For method validation, standard solution of IAA, GA₃, and TZn were flushed into mobile phase on auto sampler.

Measurement of Protease Activity

Protease activity in the root or leaf tissues was observed by spectrofluorometric instrument (FLX800, BioTek) with suc-LLVY-NH-AMC (sigma fluorogenic substrate for general protease activity) at excitation wavelength 350 nm and emission wavelength 470 nm, Z-LRR-amino luciferin (sigma fluorogenic substrate for serine protease activity) at excitation wavelength 370 nm and emission wavelength 495 nm and Caspase-3 Substrate I (sigma fluorogenic substrate for cysteine protease activity) at excitation wavelength 355 nm and emission wavelength 485 nm. Reaction mixture was made by adding 50 µg ground sample (leaf or root) into 220 µL proteolysis buffer (2 mM ATP, 10 mM KCl, 100 mM HEPES-KOH, 5 mM MgCl₂, pH adjusted at 7.5). The general protease activity was monitored through release of amino-methyl-coumarin (AMC), luciferin, and Capase-3 at result of reaction of sample and substrate at every 2 min and average was obtained.

Quantification of Phytoalexins in maize

Phytoalexins commonly found in maize under HR (zealexin A4, kauralexin A4, DIMBOA ((2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) and HDMBOA (4,7-dimethoxy-2-{[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-3,4-dihydro-2H-1,4-benzoxazin-3-one)) (Block et al., 2019) (Yang et al., 2019) were quantified using HPLC (Thermofisher scientific) which was coupled with MS (UltiMate 3000 HPLC) with a software (Thermo Xcalibur software 2.10) (Fu et al., 2018). RE or LE (2 mL) was mixed into extraction buffer (10 mL) made of HCl 1:2:0.002 H₂O::2-propanol 37 %, (v/v/v) and then vortexed for 25 sec. After rigorously shaking, first DCM (dichloromethane) was mixed with the extraction buffer in 2:1 ratio (extraction buffer :DCM). Both samples (RE or LE) were centrifuged for 40 minutes at 6077 rcf. Lower phase was taken and transferred with Pasteur pipette to Pyrex glass culture tubes. The samples were concentrated using N₂ flow (10 bar) and later heated at 42 °C. The

dried samples (2 mg) was then mixed in 2 mL of methanol in water (95.9 %). The samples were filtered by spin-X centrifuge tube at 10,000 x g.

The samples (phytoalexins extracted) were then exposed to HPLC coupled MS by injecting 20 µl sample into HPLC (Ultimate 3000) armed with a Reverse-phase column (Acclaim120 C18) at 35 °C with flow rate 0.2 mL/min. The mobile phase used was prepared by adding 0.1% formic acid in water (solvent A), and 0.1% formic acid with methanol (solvent B). Solvent gradients were collected at different time duration. The elute was deducted at 1 hour (retention time). The levels of phytoalexins was then measured by electrospraying the HPLC effluent into the mass spectrometer (Orbitrap XL) with R = 30,000. Measurements were collected at mass range (m/z 110-460) with the settings (+ive ionization mode, 4.7 kV, 280 °C capillary temperature, 35 au sheath gas flow rate and 28 au Aux Gas flow rate). For method validation, known quantities of the standard phytoalexins to RE and LE were analyzed using HPLC coupled MS (Ding et al., 2018).

Electrolytic leakage

Electrolytic leakage (EL) was measured as described (Orrego et al., 2019). Briefly, 0.3 g of leaf from every individual plant was washed with deionized water and then placed in 15 mL of falcon tube containing 15 mL of deionized water. The samples were incubated for 2 hours at 25 °C (13) and the electrolytic conductivity of the sample (L_1) was recorded. Samples were then autoclaved at 120 °C for 20 minutes, cooled down to 25 °C and the EC (L_2) was measured again.

The final read was obtained using the formula:

$$EL (\%) = \frac{L_1}{L_2} \times 100$$

Determination of marker genes involved in HR at GA₃ cell signaling perception and repression

Data available for GA₃ cell signaling perception and repression under host-microbe interaction at Expression Angler 2016 (<http://bar.utoronto.ca/ExpressionAngler/>) of BAR were extracted using the co-related gene expression with r-cut off range from 0.7 to 1.0 and -0.1 to -0.7 (Holland and Jez, 2018). The reference microbes used to extract the data were *Pseudomonas syringae* vs tomato DC3000, a bacterial hemibiotroph (Narusaka and Narusaka, 2017), *Botrytis cinerea* a fungal necrotroph (Petrasch et al., 2019), *Phytophthora infestans*, a fungal hemibiotroph (Zuluaga et al., 2016) and *Erysiphe orontii*, a fungal biotroph (Bheri et al., 2019) to obtain the universal marker genes at GA₃ cell signaling perception and repression in the above mentioned r-cut off range. Using yED Graph Editor, raw data was formatted and visualized (Reissmann and Muddukrishna, 2018). In such visualization, red balls indicated the upregulating genes with the subject gene (*GID1* or *RGR1*) and blue balls represented downregulating gene. The role of each co-expressed genes significant at our subject study was determined on TAIR (The Arabidopsis Information Resource) (www.arabidopsis.org) (Consortium et al., 2019). Homologues of 9 marker genes expressed in *Arabidopsis thaliana* were obtained in maize seedling using maize genome database (www.maizegdb.org) (Portwood et al., 2018). The expression of such 9 genes were analyzed using qRT-PCR technique in the root sample of 11 days old seedlings of maize.

Extraction of RNA and subsequent qRT-PCR analysis

Spectrum Plant Total RNA Kit (Sigma Aldrich) was added into the host root sample for RNA extraction. DNase I was also mixed to remove any genomic DNA in the sample during RNA extraction (Yin et al., 2016). Primers (Table S1) of oligo (dT)s with Super-Script III First Strand Synthesis SuperMix (Invitrogen) with s added to generate cDNA library (Hoege et al., 2017). The qRT-PCR invitrogen 1x SYBR Green I was done in a CFX96 Real time PCR (Bio-Rad detection system) (Amaral et al., 2017). In order to normalise the expression level of target genes, expression of housekeeping genes (*DPP9*) was compared (Amaral et al.,

2017). The primers for genes (Table S1) were designed using online *Oli2go* (<http://oli2go.ait.ac.at/>) on desired temperature and primer length (Hendling et al., 2018).

Table S1: List of primers used for the expression of 9 markers gene in maize seedlings

S. No.	Genes in <i>A. thaliana</i>	Homologue of Maize Gene (ID)	Primers
1.	<i>GID1</i>	<i>Zm00001d010308</i>	Forward TTCTTCTGTTGGGATGCGCC Reversed GGATTGAGCCCAAAGGGGAA
2.	<i>RGA1</i>	<i>Zm00001d041362</i>	Forward GTTGCTTGAGCCTTGTCAGC Reversed AACCTGGCAGCGATGCTATT
3.	<i>WII2</i>	<i>Zm00001d002065</i>	Forward GCAACCTGGGTGGTCGATTA Reversed ACATTGTGCGCGAAAGCAAA
4.	<i>TRX5</i>	<i>Zm00001d002690</i>	Forward ACTCCTTCCAGAACCACCTCC Reversed GGAAGGCAAGGCTGATACTGT
5.	<i>PTF1</i>	<i>Zm00001d045046</i>	Forward CAGACTGAGGTGCCAAACCT Reversed AACTGGCATCAGGCAGAGTC
6.	<i>DPMS2</i>	<i>Zm00001d034682</i>	Forward GCTGACTAGCAGAGGTGCAA Reversed AGCACGGTGCATACCTCTTC
7.	<i>TAT3</i>	<i>Zm00001d053107</i>	Forward CTTCCAGGTAGTGCACAGCA Reversed AGAAGGAGCGATACCTGGCA
8.	<i>WRKY15</i>	<i>Zm00001d036542</i>	Forward GCTAGGTTCTGTGCGTGTCT Reversed GCTCCACGAGTTCTTCACGA
9.	<i>ga2ox2</i>	<i>Zm00001d007894</i>	Forward CAACATTGGCGACACCTTCG Reversed TCTGCGTGAACGAGGAAC

RESULTS

Growth promotion under BIPOL inoculation and GA₃ inhibition

Growth promotion of plants under endophytic interaction is evaluated by RGR (Vasseur et al., 2018) and NAR (Koch et al., 2019). We recorded RGR and NAR of the maize seedlings under interaction of BIPOL at host root with SD (OD_{0.2} or OD_{0.4} or OD_{0.6}) at high or low host GA₃ level. GA₃ in host plant host was inhibited by pre-treated the host with uniconazole at leaf (**Figure 2A-2B**). Recorded data revealed that growth was promoted once GA₃ was inhibited after BIPOL inoculation at SD (OD_{0.6}) (**Figure 1C & 1F**). However, the growth was found to be very low at uniconazole treatment till 72 hour, while later restored (**Figure 1A & 1D**). Surprisingly, when we challenged the host with BIPOL alone at SD (OD_{0.6}), the growth of seedlings became highly compromised (**Figure 1B & 1E**). Moreover, when we treated the seedlings with BIPOL SD (OD_{0.2} or OD_{0.4}), the seedlings did not respond to both SDs (**Figure S1A & S1C**). Similarly, the both SDs also could not promote growth at uniconazole pre-treated seedlings when maize seedlings were at stress till 72 h (**Figure S1B-S1D**).

Cell wall integrity is very essential for the host under growth promoting condition (Mirabet et al., 2018). We determined the integrity by Electrolytic leakage (EL) as high EL value refers to host cell wall stability (Mihailova et al., 2018). As expected, EL was low in U-B seedlings at SD (OD_{0.6}) (**Figure 1I**). However, the uniconazole pre-treated seedlings of maize had high EL till 72 h which later became same as control (**Figure 1G**). As opposed, EL was very high in BIPOL treated seedlings of maize at SD (OD_{0.6}) (**Figure 1H**). Similarly, there found no change observed in EL at SD (OD_{0.2} or OD_{0.4}) in control or with pre-treatment of uniconazole to maize seedlings (**Figure S1E-S1F**).

Hormonal cross talks under BIPOL interaction with host

The interacting microbe alters the certain specific host hormonal level to successfully colonize in the host tissues (Chagas et al., 2018). Such altered hormone level produced a cross talk with other hormones which in turn produce a HR in host (Bürger and Chory, 2019). In case of BIPOL interaction with host, we observed essential hormonal cross talks of GA₃, IAA and TZn levels treated seedlings. When we inhibited GA₃ level in host by treating with uniconazole at leaf till 72 hr, the level of IAA and TZn was not affected (**Figure 2A-2B, 2F-2G, 2K-2L**). However, after 72 h, the U seedlings restored GA₃ (**Figure 2C-2E**). However, the host treated with BIPOL SD (OD_{0.6}) triggered high GA₃ level (**Figure 2A-2E**). Meanwhile the IAA and TZn was observed very low (**Figure 2F-2O**). Interestingly, in U-B seedlings, the level of IAA and TZn remained high with low GA₃ level during all treatment hours (**Figure 2A-2O**). Additionally, the SD (OD_{0.2} or OD_{0.4}) could not triggered high GA₃ in host thus the level of IAA and TZn was optimal as control (**Figure S2A-S2F**). Similarly, in GA₃ inhibited seedlings (U seedlings), the SD (OD_{0.2} or OD_{0.4}) also could not restore GA₃ (**Figure S2A-S2F**).

Secondary metabolites in host under BIPOL interaction

Secondary metabolites are produced in plants to avoid deleterious effect of biotic and abiotic stresses at the expense of plant growth and development (Yang et al., 2018). In plants, flavonoids (Tohge et al., 2018) and proline (Silva et al., 2018) are the two important group of secondary metabolites which are induced in plant under stress condition. We determined total flavonoid content and proline in LE and RE of maize through spectrophotometry technique (Zhou et al., 2018). The results showed that LE and RE had low contents of the secondary metabolites (total flavonoids and proline) in growth promoted U-B seedlings till 120h at SD (OD_{0.6}) (**Figure 3A-3J**). Conversely, the concentration of total flavonoids and proline in the growth compromised BIPOL seedlings (OD_{0.6}) was high during the same duration (**Figure 3A-3J**). However, in uniconazole treated seedlings, the metabolites were initially increased

till 72 h (**Figure 3A-3C & 3F-3G**), followed by a significant drop in the later hours (**Figure 3D-3E & 3I-3J**). Additionally, SD (OD_{0.2} or OD_{0.4}) could not elicit the production of total flavonoid contents and proline (**Figure S3A-S3B & 3E-3F**). Similarly, these SDs also could not reduce the two group of secondary metabolites in 72 h in pre-treated U seedlings (**Figure S3C-S3D & 3G-3H**).

We also quantified phenylpropanoids and glucosinolates in the treatment condition through GC/MS technique by taking ferulic acid and indolyl glucosinolate as internal standards respectively. Phenylpropanoids and glucosinolates are the group of secondary metabolites produced in plants upon the exposure to various biotic or abiotic stresses (Bhatla, 2018). These compounds aid the plant to cope with the environmental stresses (Obata, 2019). As expected from **Figure 3K-3T**, uniconazole treatment triggered high amount of phenylpropanoids and Glucosinolates till 72 h while later became less. BIPOL treatment SD (OD_{0.6}) induced highest amount of these two compounds (**Figure 3K-3T**). Interestingly, the U-B seedlings SD (OD_{0.6}) triggered low amount of these two groups of compounds (**Figure 3K-3T**). However, the amount of these two groups of compounds were same as control in SD (OD_{0.2} or OD_{0.4}) (**Figure S3I-S3J & S3M-S3N**). Similarly, at UNI treatment, the both SD (OD_{0.2} or OD_{0.4}) could not lowered the amount of phenylpropanoids and Glucosinolates (**Figure S3G-S3L & S3O-S3P**).

Enzymatic activity under BIPOL inoculation

We determined different enzymatic activities in the LE and RE maize seedlings which are operated under various stress condition (Luis et al., 2018). Two well-known enzymatic activity in host is catalase and oxidase enzymatic activities (Zheng et al., 2018). Catalase activity is severally decreased upon the plant exposure to biotic or abiotic stresses (Farooq et al., 2019). The catalase activity was low in the uniconazole treated seedlings of maize till 72 hours which later decreased (**Figure 4A-4E**). In U-B seedlings of maize at SD OD_{0.6}, the

catalase activity remained high (**Figure 4A-4E**). However, in BIPOL treated seedlings of maize SD ($OD_{0.6}$), the catalase activity was very low till 120 hours (**Figure 4A-4E**). Additionally, BIPOL inoculation SD ($OD_{0.2}$ or $OD_{0.4}$) could not increase catalase activity (**Figure S3A-S3B**). Similarly, the both SD ($OD_{0.2}$ or $OD_{0.4}$) also could not decreased catalase activity in uniconazole treated seedlings of maize till 72 hours (**Figure S3C-S3D**).

Oxidase activity is induced upon the elevated stress condition on plant (Joshi et al., 2018). As expected, uniconazole treatment to maize seedlings increased oxidase activity till 72 hour which later became normal as control. As opposed to this, the oxidase activity was low in U-B seedlings of maize at SD ($OD_{0.6}$) (**Figure 4A-4E**). Interestingly, the BIPOL inoculation SD ($OD_{0.6}$) also increased oxidase activity till 120 hours in maize seedlings (**Figure 4A-4E**). Additionally, the BIPOL inoculation at SD ($OD_{0.2}$ or $OD_{0.4}$) could not induced oxidase activity (**Figure S4E-S4F**). Similarly, both SD ($OD_{0.2}$ or $OD_{0.4}$) also could not reduce oxidase activity in uniconazole treated seedlings of maize (**Figure S4E-S4F**).

During stress condition on plant, certain enzymatic co-factors shot up in the leaf and also exuded from the plant roots in a growth medium (Speijer, 2019). We determined the reduction and induction of two major sample co-factors (NAD^+ , FAD^+) involved in this process in percent through spectrophotometric technique. As indicated from **Figure 4K-4T**, the co-factors were highly induced under uniconazole treatment in maize seedling till 72 hour which later decreased to control. BIPOL inoculation SD ($OD_{0.6}$) triggered high production of these factors till 120 hours. Conversely, the U-B treatment SD ($OD_{0.6}$) reduced the concentration of co-factors (**Figure 4K-4T**). The concentration of these enzymatic co-factors under BIPOL inoculation ($OD_{0.2}$ or $OD_{0.4}$) was same as control (**Figure S4I-S4J & S4M-S4N**). Similarly, both SDs also could not reduce the elevated level of enzymatic co-factors in U seedlings of maize (**Figure S3K-S3L & S3O-S3P**).

Hypersensitive inducing biomolecules under BIPOL inoculation

Certain undesired microbe interaction with plant host produced hypersensitive response which can be determined by the production of hypersensitive inducing molecules in plants (Terrón-Camero et al., 2018). We quantified two intracellular signalling molecules for inducing HR (c-di-GMP (Terrón-Camero et al., 2018) and cAMP level (Almblad et al., 2019) and four metabolites (phosphatidic acid (PA) pure Oxo-phytodeinoic acid (OPDA), esterified OPDA, and Jasmonic acid (JA) in maize seedlings through GC/MS technique (Genva et al., 2019). Result deducted that under uniconazole treatment, the concentration of these hypersensitive molecules was same as control (**Figure 5A-5J & 6A-6T**). Contrary, in BIPOL inoculation SD (OD_{0.6}), the concentration shot up and reached at their peaks. However, in U-B seedlings of maize the concentration remained very low (**Figure 5A-5J & 6A-6T**). Similarly, there occurred no change in the concentration of these molecules under BIPOL inoculation SD (OD_{0.2} or OD_{0.4}) in pre-treated uniconazole seedlings of maize or without its pre-treatment (**Figure S5A-S5L**).

In dying cell, the protease activity is accelerated to increase auto-digestion of the organelles in the host cell (Srikantam and Arumugam, 2019). We determined four sample protease activities (serine protease activity, cysteine proteases, and universal protease activity) in the maize seedlings under the treatment using fluorescent spectrophotometer. Results deducted from **Figure 7A-7O** that uniconazole treatment could not cause any protease activity in the maize seedlings. However, the BIPOL treatment SD (OD_{0.6}) triggered high protease activity. As opposed, the U-B seedlings of maize SD (OD_{0.6}) had lowered protease activity (**Figure 7A-7O**). In addition to this, SD (OD_{0.2} or OD_{0.4}) could not high trigger protease activity. Similarly, the BIPOL inoculation SD (OD_{0.2} or OD_{0.4}) also could not produce high protease activity in uniconazole pre-treatment to the maize seedlings (**Figure S5M-S5R**).

Upon the initiation of HR in host plant, there occurred cell death mediated by high amount of phytoalexins in the dying cell (Pitsili et al., 2019). We inspected four sample phytoalexins commonly found (zealexin A4, kauralexin A4, DIMBOA and HDMBOA) in maize seedlings (Block et al., 2019) (Yang et al., 2019). As shown in **Figure 8A-8T**, the concentration of these four phytoalexins remained unchanged in uniconazole treatment to maize seedlings. Contrary, in BIPOL inoculation SD ($OD_{0.6}$), these occurred high amount of phytoalexins (**Figure 8A-8T**). In opposed, the sample phytoalexins were noted extremely low in U-B seedling SD ($OD_{0.6}$). However, in BIPOL inoculation SD ($OD_{0.2}$ or $OD_{0.4}$), there occurred no changed with or without uniconazole treatment to maize seedlings (**Figure S5S-S5X**).

Interfering activity of GA₃ signalling under BIPOL inoculation

We observed the interfering activity of GA₃ cell signal perception and cell signal repression using co-expression data of GA₃ perception and repression during host-microbe interaction (**Figure 9A**). The description of the genes were extracted from online Arabidopsis database available (TAIR www.arabidopsis.org). The principle genes included in this case were *WII2* and *TRX5*. The product of *WII2* and *TRX5* increased the redox potential in the host cell, thus discouraged the microbial interaction. Similarly, the co-expression of *SNF7*, *EDA16*, *ALA3* and *PTF1* decreased the vacuolar transportation in the host cell to reduce fungal colonization on plant tissues. Moreover, in the same manner other cell stabilizing activities such electron transfer activity, cell apoptosis and modification of sugar molecules to form microbe resistant biomolecules were also induced by *SAG14*, *RSW10*, *PEP12* and *ADT5* expression (**Figure 9A**).

Additionally, the co-expression of upregulated DELLA (*RGAI*) determined the high expression of *DPMS2*, whose product eliminated the oxidative burst to facilitate microbe interaction (Figure 2b). Similarly, the products of *TAT3*, *SPE3* and *LOL3* commenced the

aminotransferase like enzymatic activity to aid in microbe colonization with the host. Other transcriptional facilitating processes as *WRKY15*, *RABA1*, and *ERD2* for cytoplasmic streaming to promote the microbe interaction were also upregulated (**Figure 9B**).

We selected the 9 marker genes and analyzed its expression during qRT-PCR in 11 days old maize seedling under treatments. Results deducted from qRT-PCR indicated that expression of *WII2* (*Zm00001d002065*), *TRX5* (*Zm00001d002690*) and *PTF1* (*Zm00001d045046*) was highly reduced at U-B seedlings, while the same were upregulated at BIPOL treatment to maize seedlings (**Figure 10A-10E**). No significance difference was found in uniconazole treated seedlings of maize compared to control (**Figure 10A-10E**). As opposed to these expressions, the expression of *DPMS2* (*Zm00001d034682*), *TAT3* (*Zm00001d053107*) and *WRKY15* (*Zm00001d036542*) was high at U-B seedlings while the same was low at BIPOL treatment to maize seedlings (**Figure 10F-10J**). No significance difference was found in uniconazole treatment compared to control (**Figure 10F-10J**).

However, the expression of *GID1* (*Zm00001d010308*), *RGA1* (*Zm00001d041362*) and *ga20ox2* (*Zm00001d007894*) was remained unaffected in uniconazole treatment to maize seedlings (**Figure 10K-10O**). As opposed, the expression of *GID1* (*Zm00001d010308*) and *ga20ox2* (*Zm00001d007894*) was very high while *RGA1* (*Zm00001d041362*) was low in BIPOL treatment (**Figure 10K-10O**). The U-B seedlings of maize followed the exact opposite trend in the expression of these three genes compared to BIPOL treatment as the expression of *GID1* (*Zm00001d010308*) and *ga20ox2* (*Zm00001d007894*) was very low while the expression of *RGA1* (*Zm00001d041362*) was high (**Figure 10K-10O**).

Root colonization of BIPOL under high GA₃ concentration

Fungal colonization was noted very low for BIPOL treated seedlings at SD (OD_{0.6}), whereas it was relatively high in U-B seedlings compared to control seedlings of maize at SD (OD_{0.6})

(Figure 11A). However, there was found no change in percent colonization of BIPOL at SD (OD_{0.2} or OD_{0.4}) in both BIPOL and U-B treated seedlings of maize (Figure 11B).

Figure 1

Determination of RGR (relative growth rate), NAR (net assimilation rate), and EC (electrolytic content leakage) in the 11 days old maize seedlings exposed to different treatments including C (control), U (Uniconazole), B (BIPOL), U-B (Uniconazole-BIPOL) at spore density 0.6 for the different time duration. Duncan's test was performed and different alphabetic letters shows the significant difference. Experiment was repeated at least three time independently.

Figure: 2

Determination of GA₃ (gibberellins), TZn (trans-zeatin), and IAA (indole-3-acetic acid) in the LE (leaf extracts) and RE (root exudates) of 11 days old maize seedlings exposed to different treatments including C (control); U (Uniconazole), B (BIPOL), U-B (Uniconazole-

486 BIPOL) for the different time duration. Duncan's test was performed and different alphabetic
487 letters shows the significant difference. Experiment was repeated at least three time
488 independently.

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Figure 3

Quantification of Proline, TFC (total flavonoid content), PPs (phenylpropanoids) and GLs (Glucosynaloids) in the 11 days old maize seedlings exposed to different treatments including C (control), U (Uniconazole), B (BIPOL), U-B (Uniconazole-BIPOL) at spore

density 0.6 for the different time duration. Duncan's test was performed and different alphabetic letters shows the significant difference. Experiment was repeated at least three time independently.

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502 **Figure 4**

503 Determination of Oxidase, Catalase enzymatic activities, NAD⁺ (cofactor) and FAD⁺
 504 (cofactor) in the 11 days old maize seedlings exposed to different treatments including C
 505 (control), U (Uniconazole), B (BIPOL), U-B (Uniconazole-BIPOL) at spore density 0.6 for
 506 the different time duration. Ducan's test was performed and different alphabetic letters shows
 507 the significant difference. Experiment was repeated at least three time independently.
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510 **Figure 5**

511 Determination of phosphatidic acid (PA) pure Oxo-phytodeinoic acid (OPDA), esterified
 512 OPDA, and Jasmonic acid (JA) in the 11 days old maize seedlings exposed to different
 513 treatments including C (control), U (Uniconazole), B (BIPOL), U-B (Uniconazole-BIPOL) at
 514 spore density 0.6 for the different time duration. Duncan's test was performed and different
 515 alphabetic letters shows the significant difference. Experiment was repeated at least three
 516 time independently.

518 **Figure 6**

519 Quantification of c-di-GMP (3',5'-cyclic diguanylic acid) and cAMP (3',5'-cyclic adenosine
520 monophosphate) in the 11 days old maize seedlings exposed to different treatments including
521 C (control), U (Uniconazole), B (BIPOL), U-B (Uniconazole-BIPOL) at spore density 0.6 for
522 the different time duration. Duncan's test was performed and different alphabetic letters shows
523 the significant difference. Experiment was repeated at least three time independently.

Figure 7

Quantification of Universal protease activity, serine protease activity and cysteine protease activity in the 11 days old maize seedlings exposed to different treatments including C

528 (control), U (Uniconazole), B (BIPOL), U-B (Uniconazole-BIPOL) at spore density 0.6 for
 529 the different time duration. Duncan's test was performed and different alphabetic letters shows
 530 the significant difference. Experiment was repeated at least three time independently.
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533 Figure 8

534 Quantification of commonly found phytoalexins in maize (Zealexins A4, kauralexin A4,
 535 DIMBOA and HDMBOA) in the 11 days old maize seedlings exposed to different treatments

including C (control), U (Uniconazole), B (BIPOL), U-B (Uniconazole-BIPOL) at spore density 0.6 for the different time duration. Duncan's test was performed and different alphabetic letters shows the significant difference. Experiment was repeated at least three time independently.

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Figure 9

Data extracted from Expression Angler 2016 showing the set of co-expressed genes with *GID* (a) and DELLA (*Mihailova et al.*) (b) under microbe stresses based on R- cut off range -0.7 to 0.9) with the principle gene expression.

Figure 10

Determination of gene expression in the leaf tissues of 11 days old maize seedlings exposed to different treatments including C (control); U (Uniconazole), B (BIPOL), U-B

593 (Uniconazole-BIPOL) for the different time duration. Duncan's test was performed and
594 different alphabetic letters shows the significant difference. Experiment was repeated at least
595 three time independently.

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597

Figure 11

Determination of fungal colonization on maize root under the stress of BIPOL (BIPOL) and UNI-BIPOL (Uniconazole-BIPOL) in spore density ($OD_{0.2}$, $OD_{0.4}$ and $OD_{0.6}$) on the roots of maize seedlings. Duncan's test was performed and different alphabetic letters shows the significant difference. Experiment was repeated at least three time independently.

Discussion

HR in plant is elicited in response to interaction of the host plant with the undesirable microbe (Ger and Chang, 2019). The microbe may be a pathogen or inert to the host plant (Hatsugai et al., 2018). When we applied BIPOL to the roots of maize 11 day old seedlings in hydroponic solution (Hassan), the seedlings elicited a HR. The HR deeply affected the growth and development of the seedlings. As the result, the B seedlings had low RGR and NAR. Accompanied by this, the seedlings also deterred the colonization of BIPOL at their roots. Interestingly, the seedlings elicited HR was limited to the BIPOL SD ($OD_{0.6}$). There was no such response observed in BIPOL SD ($OD_{0.2}$ or $OD_{0.4}$). It clearly meant that the specific spore density of BIPOL could elicited a HR in host. Most of the pathogenic fungi undergo HR in host once in contact with host plant at maximum SD. We here concluded that BIPOL SD inducing HR is $OD_{0.6}$.

Most of plant hormones acting phytosignalling molecules aid the host plant to induce the HR during its interaction with undesirable microbe (Li et al., 2019). We observed high level of GA_3 in host leaf and root exudates after application of BIPOL SD ($OD_{0.6}$) to the host roots. Interestingly, this high level of GA_3 reduced the level of IAA and TZn in host leaf and root exudates which clearly indicated the high level of GA_3 produced negative cross talks with IAA and TZn at the interaction of BIPOL. We blocked the biosynthesis of GA_3 for 72 hour of treatment by applying uniconazole to the leaf of 11 days old seedlings. GA_3 is the growth plant hormones which induce cell elongation and division in maize plants (Zhang et al., 2019). Uniconazole treatment blocked GA_3 biosynthesis at product level. As expected, the growth of the maize seedlings reduced significantly, determined by low RGR and NAR values. Surprisingly, in GA_3 deficient environment, BIPOL SD ($OD_{0.6}$) colonized the host root and promoted the growth. However, BIPOL SD ($OD_{0.2}$ or $OD_{0.4}$) could not promote growth in uniconazole treated seedlings. This clearly indicated that both SDs are not effective

either at pathogenic level or growth promoting level. We also examined the growth suppression of Uniconazole treated seedlings and BIPOL SD (OD_{0.6}) treated seedling through the production of secondary metabolites in host leaf and root exudates. Plant produces secondary metabolites under biotic or abiotic stress to cope them at the expense of its normal growth and development (Yang et al., 2018). As expected, the level of Proline, TFC (total flavonoid content), PPs (phenylpropanoids) and GLs (Glucosinolates) were high in Uniconazole treated seedlings and BIPOL SD (OD_{0.6}) treated seedling. However, after the release of Uniconazole effect on the maize seedlings, the level of secondary metabolites was lowered.

Several biotic or abiotic stresses induce oxidase activity in plants which in turn decrease growth of the host plant to eliminate the stress condition (Selinski et al., 2018). As expected, the treatment of uniconazole to maize seedlings increased oxidase activity and subsequently decreased plant growth till 72 hours after which the oxidase activity became normal. However, the oxidase activity remained high in BIPOL treatment SD (OD_{0.6}) with co-related low growth rate of the seedlings. As opposed, high calatase activity was noted in prolifically growing seedlings (Poli et al., 2018). Similarly, we observed low growth in low catalase activity treatments (uniconazole till 72 hours and BIPOL SD (OD_{0.6}) in all duration). In the contrary, BIPOL treatment SD (OD_{0.6}).

On the contrary, BIPOL SD (OD_{0.6}) treated seedling could not reduce the level of secondary metabolites was lowered. This literally meant that stress of BIPOL SD (OD_{0.6}) treatment remained high during the treatment hours. Additionally, the host seedlings did not respond to BIPOL SD (OD_{0.2} or OD_{0.4}) due to normal secondary metabolites concentration in both leaf and root exudates. Similarly, the same treatments too did not reduce the high concentration of secondary metabolites in Uniconazole treated seedlings. We determined HR in host in host by quantifying the PA, pure OPDA, esterified OPDA, and JA levels in host under biotic and

abiotic stress (Schuman et al., 2018). As expected, the maize seedlings containing high concentration of GA₃ (B seedlings treated by BIPOL SD (OD_{0.6})) produced high level of HR inducing molecules while the seedlings containing low GA₃ level (U-B seedlings treated by BIPOL SD (OD_{0.2} or OD_{0.4})) produced low concentration of these molecules. However, the other two SDs could not trigger the production of HR inducing molecules in neither in Uniconazole treated seedlings nor control seedlings.

This deducted that the two BIPOL SD (OD_{0.2} or OD_{0.4})) treatment to maize seedlings are inert, neither growth promoting and virulent. The same pattern was also followed by HR signalling inducing molecules as c-di-GMP, and cAMP. In host plant under the interaction of undesirable microbe, several protease activities begin which break down the proteins involves in the transportation of nutrients and facilitation of microbial colonization (Havé et al., 2018). We determined three marker protease activities such as universal protease activity, serine protease activity and cysteine protease. The quantification of protease activities also showed high HR in the maize seedling stressed with BIPOL SD (OD_{0.6}) only. As the HR triggered in host plant due to microbe interaction, cell death inducing substances are biosynthesized known as phytoalexins (Komives and Kiraly, 2019). We determined four phytoalexins commonly found in maize as Zealexins A4, kauralexin A4, DIMBOA and HDMBOA (Yang et al., 2019). As expected, the level these phytoalexins were high only in BIPOL SD (OD_{0.6}) treatment.

Using in-silico approach, we finally extracted data regarding gene expressions in *A. thaliana* under the interaction with model biotrophs and necrotroph at GA₃ hypersensitivity. The expression of 9 markers genes were analysed in maize seedlings using qRT-PCR. The result showed that genes (*WII2*, *TRX5*, *PTF1*, *DPMS2*, *TAT3*, *WRKY15*, *GID1*, *ga20ox2* and *RGR1*) inhibiting microbe colonization in host were highly upregulated in the seedlings which had high GA₃ level (BIPOL treated seedlings). On the contrary, such genes were down regulated

in Y-B thus facilitating BIPOL interaction. The description of the genes were taken from the online available database of Arabidopsis (TAIR) (Consortium et al., 2019). After overall inspection, we also determined BIPOL colonization frequency on the roots of maize seedlings. During plant microbe interaction, roots of the most plant host provide optimal site for colonization (Hugoni et al., 2018). As expected, the BIPOL SD (OD_{0.6}) colonized only in the roots of the uniconazole pre-treated seedlings of maize at leaf. It was because the level of GA₃ was inhibited which could interfered the colonization. Due to the inhibition of GA₃, IAA and Transzeatin levels were high in the seedlings. IAA (Mehmood et al., 2018) and Tranzeatin (Kabbara et al., 2018) are involved to have a role in the accommodation of fungal colonization on host plant roots. As opposed, in the seedling of maize where GA₃ was high (control seedlings treated with BIPOL SD (OD_{0.6})), the BIPOL colonization was hindered. This could be due to the high level of GA₃ which developed cross talks with IAA and Tranzeatin, thus decreasing its production in host.

Conclusion

Beside growth inducing molecules, GA₃ also indicates the compatibility of host-micorbial interaction. Inoculation of BIPOL majorly caused GA₃ hypersensitivity at a SD (OD_{0.6}) at 600 nm. this hypersensitivity interfered the optimal level of IAA and Trans-zeatin in the host, thereby demoting growth of the seedlings. Once the GA₃ was inhibited using uniconazole treatment BIPOL SD (OD_{0.6}) colonized on the seedlings root and resumed its growth promoting acitivity. This supported our hypothesis that GA₃ hypersensitivity hinders the interaction of BIPOL with *Z. mays* under its cross talks with IAA and trans-zeatin.

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