Phytohormonal cross-talk modulate Bipolaris sorokiniana (Scc.)interaction with Zea mays

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Abstract

Besides acting as growth inducing molecule, Gibberellin (GA_3) also confers the compatibility of microbial interactions with host. We inoculated 11 days old *Z. mays* seedlings grown under hydroponic conditions and high GA_3 levels with *Bipolaris sorokiniana* (BIPOL) at the spore density (SD) of $OD_{0.6}$. The high level of GA_3 negatively affected the growth of the seedlings, accompanied by the high level of stress deducing secondary metabolites (proline, total flavanoids, phenylpropanoids, and glucosinolides). Moreover, high level of GA_3 produced a hypersensitive response (HR) in the seedlings. The HR developed cross talks with IAA and trans-zeatins and triggered higher production of hypersensitive inducing biomolecules. The other HR co-related biological processes were demonstrated by high phytoalexins level and high protease activities. Such activities ultimately inhibited the colonization of BIPOL on the roots of maize seedlings. The products of the genes expressed at high GA_3 also conferred the deterrence of BIPOL colonization at SD = $OD_{0.6}$. Intriguingly, when we inhibited GA_3 biosynthesis in the seedlings with aerially sprayed uniconizole, prior to BIPOL treatment, the BIPOL colonized and subsequently promoted the seedling growth. This low level of GA_3 after BIPOL treatment checked the high level of secondary metabolites and hypersensitivity inducing molecules. The results, thus suggested that the aforementioned processes only happened in the BIPOL at SD ($OD_{0.6}$), whereas the SD at lower levels ($OD_{0.2}$ or $OD_{0.4}$) neither promoted the growth of uniconizole pre-treated seedlings nor produced HR in control seedlings of maize plant.

Keywords: Plant-micorbes interaction, hormonal cross talks, growth activity, Uniconizole, *Zea mays*

Introduction

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

Hypersensitive response (HR) in plant is triggered upon the interaction of undesired microbe for host plant (Matić et al., 2016). HR in plant is characterized by the production hypersensitive inducing molecules such as c-di-GMP, and cAMP (Cadby et al., 2019). Other signalling molecules such as phosphatidic acid (PA), free or esterified oxo-phytodeinoic acid (OPDA), and jasmonic acid (JA) are also at the site of HR in host (Hou et al., 2016). These molecules activates the host defence response against the microbe (Lim et al., 2017). Accompanied by this, the host plant produces phytoalexins (Suárez et al., 2018) and high protease activity (Asai and Shirasu, 2015). Primarily, such HR is activated at the response of high level of phyto-hormone (Großkinsky et al., 2016). In plant, IAA, GA_3 and trans-zeatin are responsible for the HR (Body et al., 2019).

During HR to any stress, the host plant growth is adversely affected which is optimally be determined by Relative Growth Rate (RGR) (Vasseur et al., 2018) and Net Assimilation Rate (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 supress the E3 ligase
69 polyubiquitination to inhibit GA_3 from degrading DELLA protein (Li et al., 2019). Most of
70 the arbscular fungi (AF) are known to utilize GA_3 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 GA_3 and high DELLA protein activity (McGuiness 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 GA_3 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 GA_3 in host plants
80 also interferes with the other phytohormones (Tijero et al., 2019). GA_3 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 GA_3 thus interferes with the growth and development of the host plants
84 through establishing of cross talks between GA_3 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 GA_3 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 GA_3 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 ºC for 21 days for vernalisation period (Müller et al., 2017). At the time of experiment, the seeds were treated with dilute solution of $HgCl_2$ (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 ºC 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 ºC 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 ºC for 5 days in a shaking incubator operated at 150 rpm (Khan et al., 2015). On the 5^{th} 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 ºC in a shaking incubator. Prior to inoculation, the spore was washed by centrifugating at 10^5 xg at 10 ºC 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 ºC. 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

139 extraction of phytochemicals (Dhawan and Gupta, 2017). The obtained leaf extract (LE) was
140 first filtered and the filtrate was concentrated in the rotary evaporatory (Wahyuningsih et al.,
141 2017). Moreover, root exudates (RE) was obtained from filtered hypdroponic culture of the
142 root seedlings and concentrated in the rotary evaporatory.

143 **Secondary metabolite determination**

144 Colorimetric method was used for the evaluation of total flavonoids and proline contents.
145 Samples (1 mL) of LE and RE were prepared as mentioned above, mixed in 3 % sulfo-
146 salicylic acid (4 mL) and centrifuged for 5 mins at 6077 rcf. After centrifugation, the 2 mL of
147 ninhydrin reagent was added to the samples and shaken vigorously. Acid ninhydrin reagent
148 was prepared by adding 1.25 g of ninhydrin in pure glacial acetic acid (30 mL) and 20 mL of
149 phosphoric acid (6 M) and mixed. The reaction mixture was heated for 1 hour at 100 ºC and
150 the pellet found was disappeared in toluene (4 mL) (Lee et al., 2018). OD of the samples
151 were monitored using UV/Vis spectrophotometer (Lambda 1050) at 520 nm. Similarly, for
152 determination of total flavonoid contents, 0.5 mL of LE and RE samples were added into 10
153 % potassium acetate (100 µL), 10 % aluminium chloride (100 µL), 70 % ethanol (4.3 mL).
154 The mixture was incubated at room temperature for 30 minutes and the OD was measured at
155 450 nm on UV/Vis spectrophotometer (Lambda 1050) (Gil-Ramírez et al., 2016).

156 Phenylpropanoids and glucosinolates were determined using HPLC technique by
157 taking ferulic acid (Genovese et al., 2018) and indolyl glucosinolates (Aghajanzadeh et al.,
158 2019) as standards respectively. The samples (RE or LE) first were filtered through a 0.2 µm
159 membrane filter associated in a syringe filtration. The filtrated sample (20µL) was then
160 poured into a C18 reverse phase HPLC column and eluted with 75 % methanol (in % 10
161 acetic acid) for Phenylpropanoids and glucosinolides through an isocratic pump. The eluate
162 was monitored through a UV detector set at 212 nm and 287 nm for Phenylpropanoids and
163 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 µL) was mixed with 1.5 mL of phosphate buffer (50 mM), 200 µL of ascorbic acid (0.5 mM), and 200 µL of H_2O_2 (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 µL sample with 400 µL of H_2O_2 (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 µL $MgCl_2$ (500 mM), Tris-HCl Buffer (0.5 mL) and 100 µL 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 µL ascorbic acid (250 mM) and 100 µL FADH. Reaction was initiated by adding Metolachlor OA ($C_{15}H_{21}NO_4$). 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), GA_3 (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, GA_3, 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, GA_3, and TZn were flushed into mobile phase on auto sampler.

214 **Measurement of Protease Activity**

215 Protease activity in the root or leaf tissues was observed by spectrofluorometric instrument
216 (FLX800, BioTek) with suc-LLVY-NH-AMC (sigma fluorogenic substrate for general
217 protease activity) at excitation wavelength 350 nm and emission wavelength 470 nm, Z-LRR-
218 amino luciferin (sigma fluorogenic substrate for serine protease activity) at excitation
219 wavelength 370 nm and emission wavelength 495 nm and Caspase-3 Substrate I (sigma
220 fluorogenic substrate for cysteine protease activity) at excitation wavelength 355 nm and
221 emission wavelength 485 nm. Reaction mixture was made by adding 50 µg ground sample
222 (leaf or root) into 220 µL proteolysis buffer (2 mM ATP, 10 mM KCl, 100 mM HEPES-
223 KOH, 5 MM $MgCl_2$, pH adjusted at 7.5). The general protease activity was monitored
224 through release of amino-methyl-coumarin (AMC), luciferin, and Capase-3 at result of
225 reaction of sample and substrate at every 2 min and average was obtained.

226 **Quantification of Phytoalexins in maize**

227 Phytoalexins commonly found in maize under HR (zealexin A4, kauralexin A4, DIMBOA
228 ((2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) and HDMBOA (4,7-dimethoxy-2-{[3,4,5-
229 trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-3,4-dihydro-2H-1,4-benzoxazin-3-one))
230 (Block et al., 2019) (Yang et al., 2019) were quantified using HPLC (Thermofisher scientific)
231 which was coupled with MS (UltiMate 3000 HPLC) with a software (Thermo Xcalibur
232 software 2.10) (Fu et al., 2018). RE or LE (2 mL) was mixed into extraction buffer (10 mL)
233 made of HCl 1:2:0.002 H_2O::2-propanol 37 %, (v/v/v) and then vortexed for 25 sec. After
234 rigorously shaking, first DCM (dichloromethane) was mixed with the extraction buffer in 2:1
235 ratio (extraction buffer :DCM). Both samples (RE or LE) were centrifuged for 40 minutes at
236 6077 rcf. Lower phase was taken and transferred with Pasteur pipette to Pyrex glass culture
237 tubes. The samples were concentrated using N_2 flow (10 bar) and later heated at 42 $^{\circ}$C. The

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

240 The samples (phytoalexins extracted) were then exposed to HPLC coupled MS by
241 injecting 20 µl sample into HPLC (Ultimate 3000) armed with a Reverse-phase column
242 (Acclaim120 C18) at 35 °C with flow rate 0.2 mL/min. The mobile phase used was prepared
243 by adding 0.1% formic acid in water (solvent A), and 0.1% formic acid with methanol
244 (solvent B). Solvent gradients were collected at different time duration. The elute was
245 deducted at 1 hour (retention time). The levels of phytoalexins was then measured by
246 electrospraying the HPLC effluent into the mass spectrometer (Orbitrap XL) with R =
247 30,000. Measuremnts was collected at mass range (m/z 110-460) with the settings (+ive
248 ionization mode, 4.7 kV, 280 °C capillary temperature, 35 au sheath gas flow rate and 28 au
249 Aux Ga flow rate). For method validation, known quantities of the standard phytoalexins to
250 RE and LE were analyzed using HPLC coupled MS (Ding et al., 2018).

251 **Electrolytic leakage**

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

258 The final read was obtained using the formula:

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

259 **Determination of marker genes involved in HR at GA_3 cell signaling perception and**
260 **repression**

Data available for GA_3 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 GA_3 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 (dTs) with Super-Script III Frist 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.,

286 2017). The primers for genes (Table S1) were designed using online *Oli2go* (

287 http://oli2go.ait.ac.at/) on desired temperature and primer length (Hendling et al., 2018).

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

S. No.	Genes in *A. thaliana*	Homologue of Maize Gene (ID)	Primers
1.	*GID1*	*Zm00001d010308*	Forward TTCTTCTGTTGGGATGCGCC Reversed GGATTGAGCCCAAAGGGGAA
2.	*RGA1*	*Zm00001d041362*	Forward GTTGCTTGAGCCTTGTCAGC Reversed AACCTGGCAGCGATGCTATT
3.	*WII2*	*Zm00001d002065*	Forward GCAACCTGGGTGGTCGATTA Reversed ACATTGTGCGCGAAAGCAAA
4.	*TRX5*	*Zm00001d002690*	Forward ACTCCTTCCAGAACCACCTCC Reversed GGAAGGCAAGGCTGATACTGT
5.	*PTF1*	*Zm00001d045046*	Forward CAGACTGAGGTGCCAAACCT Reversed AACTGGCATCAGGCAGAGTC
6.	*DPMS2*	*Zm00001d034682*	Forward GCTGACTAGCAGAGGTGCAA Reversed AGCACGGTGCATACCTCTTC
7.	*TAT3*	*Zm00001d053107*	Forward CTTCCAGGTAGTGCACAGCA Reversed AGAAGGAGCGATACCTGGCA
8.	*WRKY15*	*Zm00001d036542*	Forward GCTAGGTTCTGTGCGTGTCT Reversed GCTCCACGAGTTCTTCACGA
9.	*ga20ox2*	*Zm00001d007894*	Forward CAACATTGGCGACACCTTCG Reversed TCTGCGTGAACTCGAGGAAC

289

290

RESULTS

Growth promotion under BIPOL inoculation and GA_3 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 GA_3 level. GA_3 in host plant host was inhibited by pre-treated the host with uniconizole at leaf (**Figure 2A-2B)**. Recorded data revealed that growth was promoted once GA_3 was inhibited after BIPOL inoculation at SD ($OD_{0.6}$) (**Figure 1C & 1F**). However, the growth was found to be very low at uniconizole 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 uniconizole 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 uniconizole 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 uniconizole to maize seedlings (**Figure S1E-S1F**).

Hormonal cross talks under BIPOL interaction with host

315 The interacting microbe alters the certain specific host hormonal level to successfully
316 colonize in the host tissues (Chagas et al., 2018). Such altered hormone level produced a
317 cross talk with other hormones which in turn produce a HR in host (Bürger and Chory, 2019).
318 In case of BIPOL interaction with host, we observed essential hormonal cross talks of GA_3,
319 IAA and TZn levels treated seedlings. When we inhibited GA_3 level in host by treating with
320 uniconizole at leaf till 72 hr, the level of IAA and TZn was not affected (**Figure 2A-2B, 2F-**
321 **2G, 2K-2L**). However, after 72 h, the U seedlings restored GA_3 (**Figure 2C-2E**). However,
322 the host treated with BIPOL SD ($OD_{0.6}$) triggered high GA_3 level (**Figure 2A-2E**).
323 Meanwhile the IAA and TZn was observed very low (**Figure 2F-2O**). Interestingly, in U-B
324 seedlings, the level of IAA and TZn remained high with low GA_3 level during all treatment
325 hours (**Figure 2A-2O**). Additionally, the SD ($OD_{0.2}$ or $OD_{0.4}$) could not triggered high GA_3 in
326 host thus the level of IAA and TZn was optimal as control (**Figure S2A-S2F**). Similarly, in
327 GA3 inhibited seedlings (U seedlings), the SD ($OD_{0.2}$ or $OD_{0.4}$) also could not restore GA_3
328 (**Figure S2A-S2F**).

329 **Secondary metabolites in host under BIPOL interaction**

330 Secondary metabolites are produced in plants to avoid deleterious effect of biotic and abiotic
331 stresses at the expense of plant growth and development (Yang et al., 2018). In plants,
332 flavonoids (Tohge et al., 2018) and proline (Silva et al., 2018) are the two important group of
333 secondary metabolites which are induced in plant under stress condition. We determined total
334 flavonoid content and proline in LE and RE of maize through spectrophotometry technique
335 (Zhou et al., 2018). The results showed that LE and RE had low contents of the secondary
336 metabolites (total flavonoids and proline) in growth promoted U-B seedlings till 120h at SD
337 ($OD_{0.6}$) (**Figure 3A-3J**). Conversely, the concentration of total flavonoids and proline in the
338 growth compromised BIPOL seedlings ($OD_{0.6}$) was high during the same duration (**Figure**
339 **3A-3J**). However, in uniconizole 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**, uniconizole 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 uniconizole 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

365 catalase activity remained high (**Figure 4A-4E**). However, in BIPOL treated seedlings of
366 maize SD ($OD_{0.6}$), the catalase activity was very low till 120 hours (**Figure 4A-4E**).
367 Additionally, BIPOL inoculation SD (OD_{02} or $OD_{0.4}$) could not increase catalase activity
368 (**Figure S3A-S3B**). Similarly, the both SD (OD_{02} or $OD_{0.4}$) also could not decreased catalase
369 activity in uniconizole treated seedlings of maize till 72 hours (**Figure S3C-S3D**).

370 Oxidase activity is induced upon the elevated stress condition on plant (Joshi et al.,
371 2018). As expected, uniconizole treatment to maize seedlings increased oxidase activity till
372 72 hour which later became normal as control. As opposed to this, the oxidase activity was
373 low in U-B seedlings of maize at SD ($OD_{0.6}$) (**Figure 4A-4E**). Interestingly, the BIPOL
374 inoculation SD ($OD_{0.6}$) also increased oxidase activity till 120 hours in maize seedlings
375 (**Figure 4A-4E**). Additionally, the BIPOL inoculation at SD (OD_{02} or $OD_{0.4}$) could not
376 induced oxidase activity (**Figure S4E-S4F**). Similarly, both SD (OD_{02} or $OD_{0.4}$) also could
377 not reduce oxidase activity in uniconizole treated seedlings of maize (**Figure S4E-S4F**).

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

389 **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 uniconizole 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 uniconizole 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 uniconizole 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 uniconizole 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 uniconizole 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 uniconizole treatment to maize seedlings (**Figure S5S-S5X**).

Interfering activity of GA_3 signalling under BIPOL inoculation

We observed the interfering activity of GA_3 cell signal perception and cell signal repression using co-expression data of GA_3 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 vacoular 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 (*RGA1*) 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 olf 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 uniconizole 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 uniconizole treatment compared to control (**Figure 10F-10J**).

However, the expression of *GID1* (*Zm00001d010308*), *RGA1* (*Zm00001d041362*) and ga20ox2 (*Zm00001d007894*) was remained unaffected in uniconizole 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 GA_3 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 ($OD_{0.6}$)

463 (**Figure 11A**). However, there was found no change in percent colonization of BIPOL at SD

464 ($OD_{0.2}$ or $OD_{0.4}$) in both BIPOL and U-B treated seedlings of maize (**Figure 11B**).

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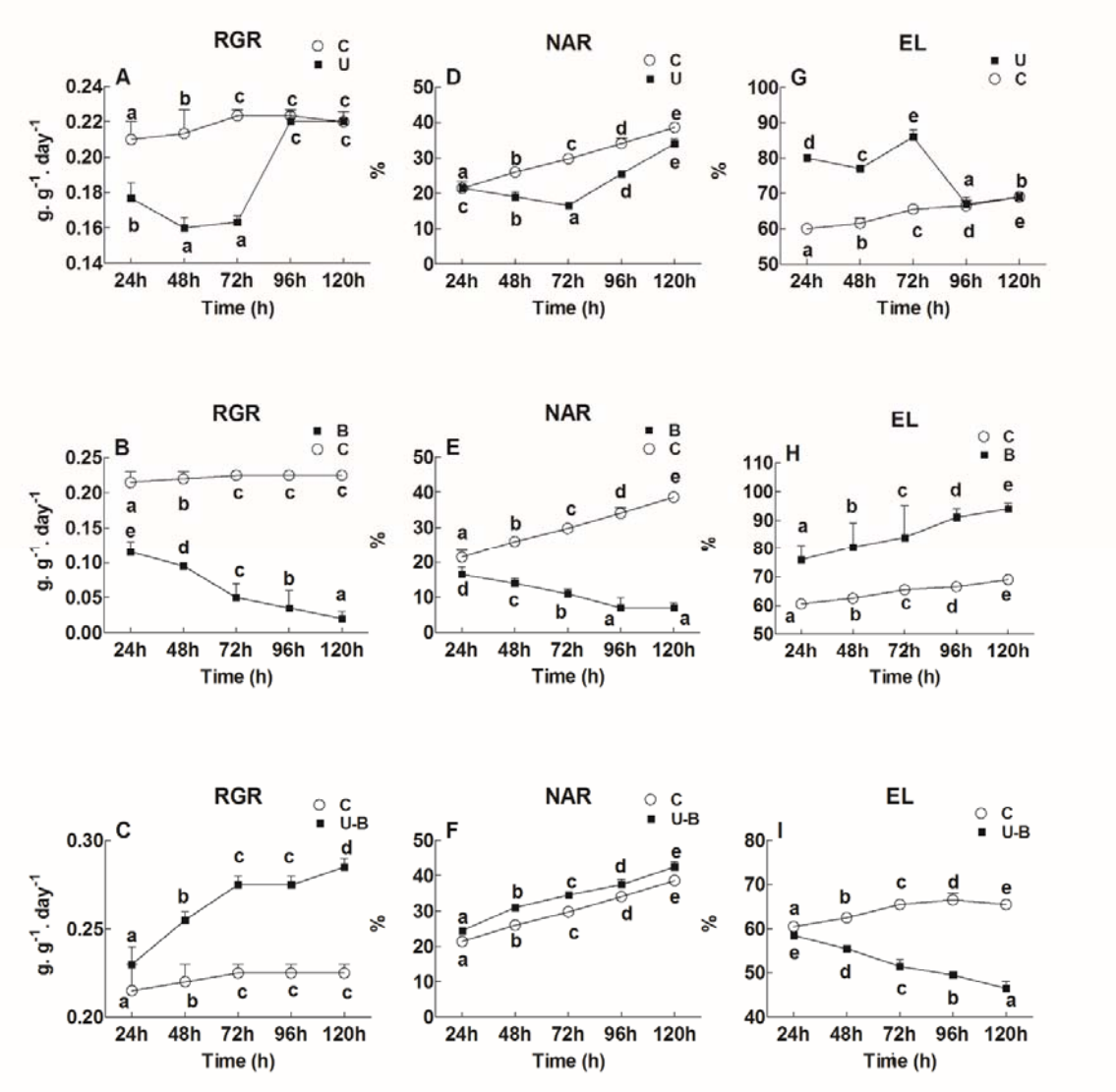

470

471 **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 (Uniconizole), B (BIPOL), U-B (Uniconizole-BIPOL) at spore density 0.6 for the different time duration. Ducan's test was performed and different alphabetic letters shows the significant difference. Experiment was repeated at least three time independently.

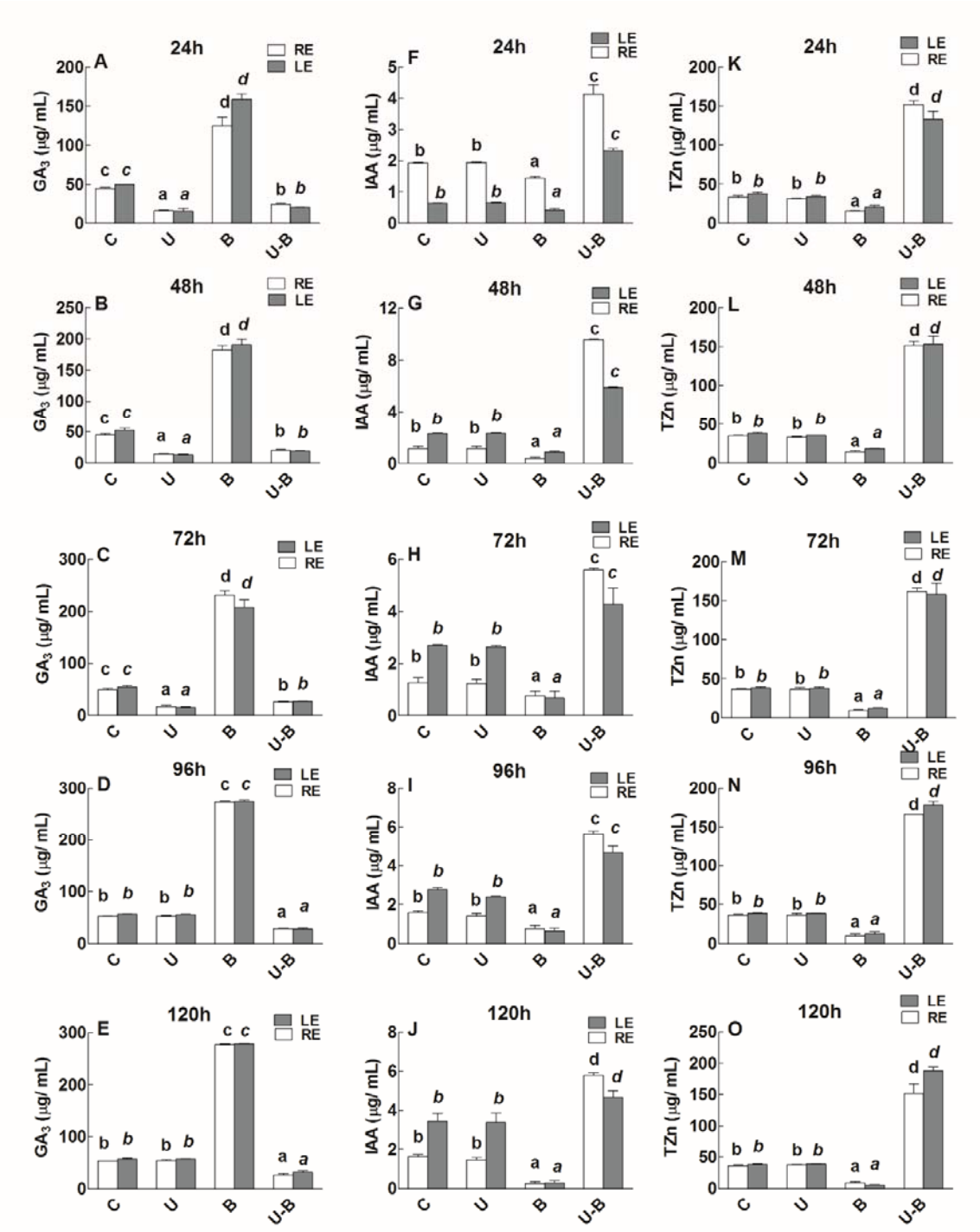

Figure: 2

Determination of GA_3 (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 (Uniconizole), B (BIPOL), U-B (Uniconizole-

BIPOL) for the different time duration. Ducan's test was performed and different alphabetic letters shows the significant difference. Experiement was repeated at least three time independently.

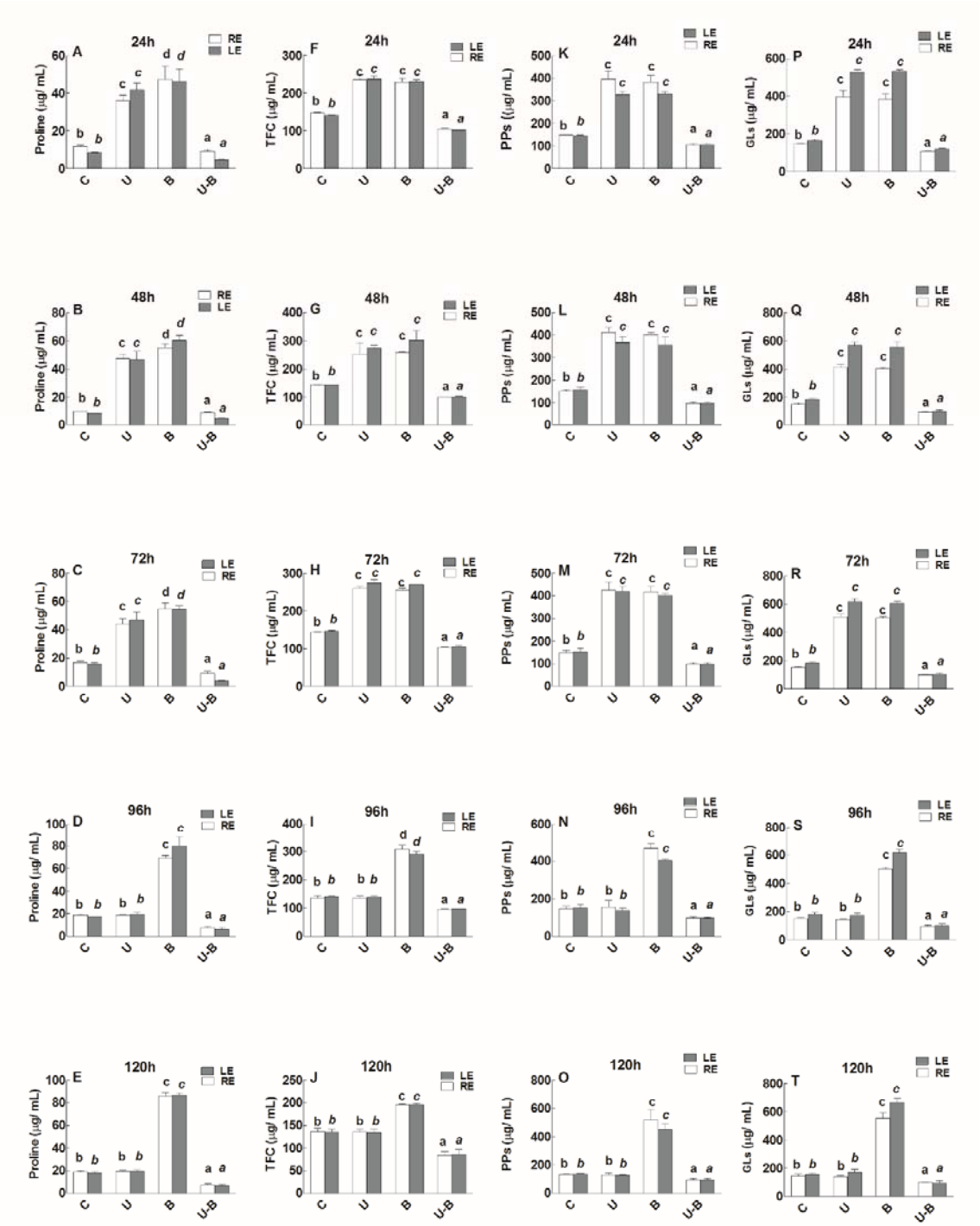

Figure 3

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 (Uniconizole), B (BIPOL), U-B (Uniconizole-BIPOL) at spore

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

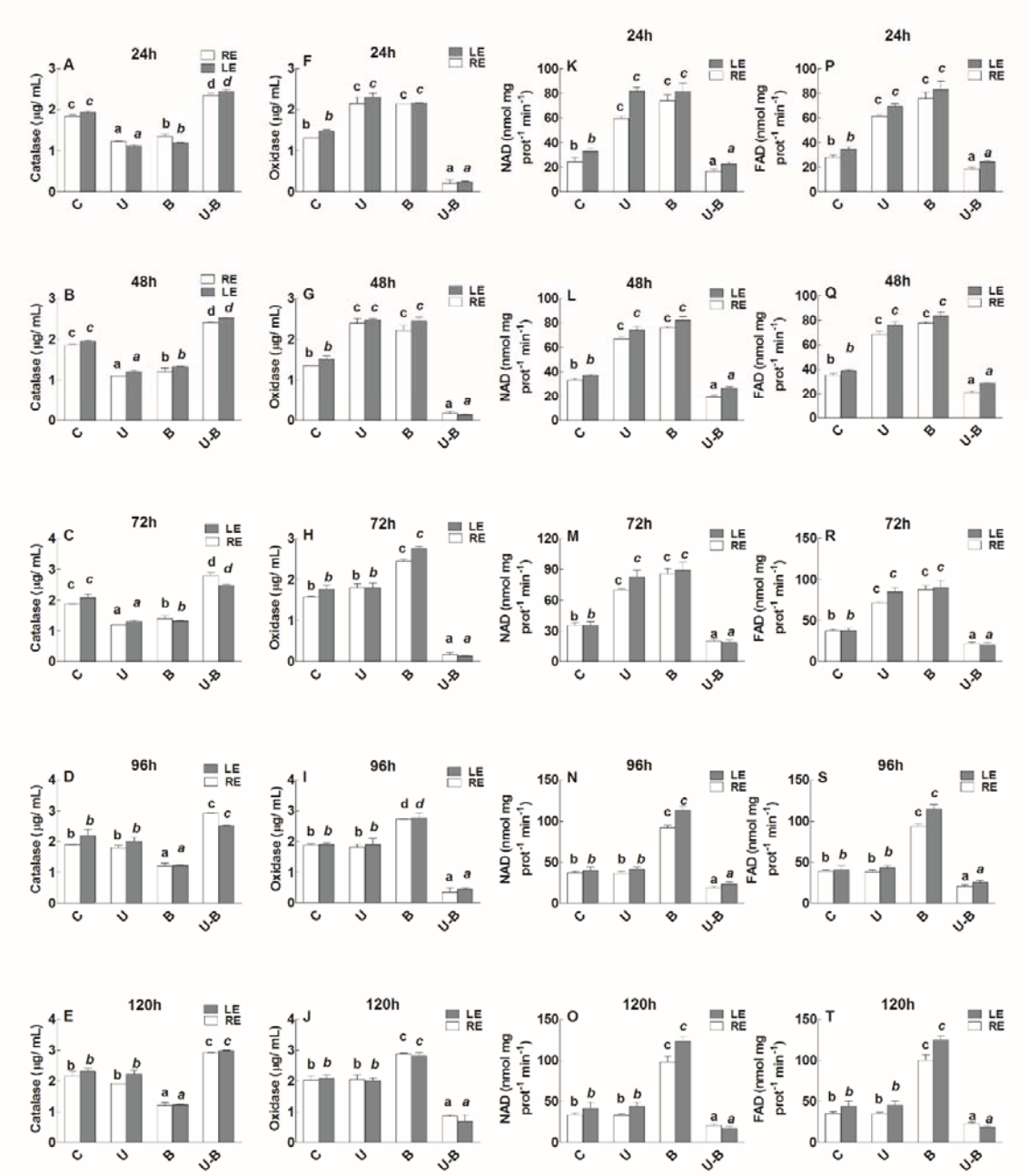

Figure 4

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 (Uniconizole), B (BIPOL), U-B (Uniconizole-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. Experiement was repeated at least three time independently.
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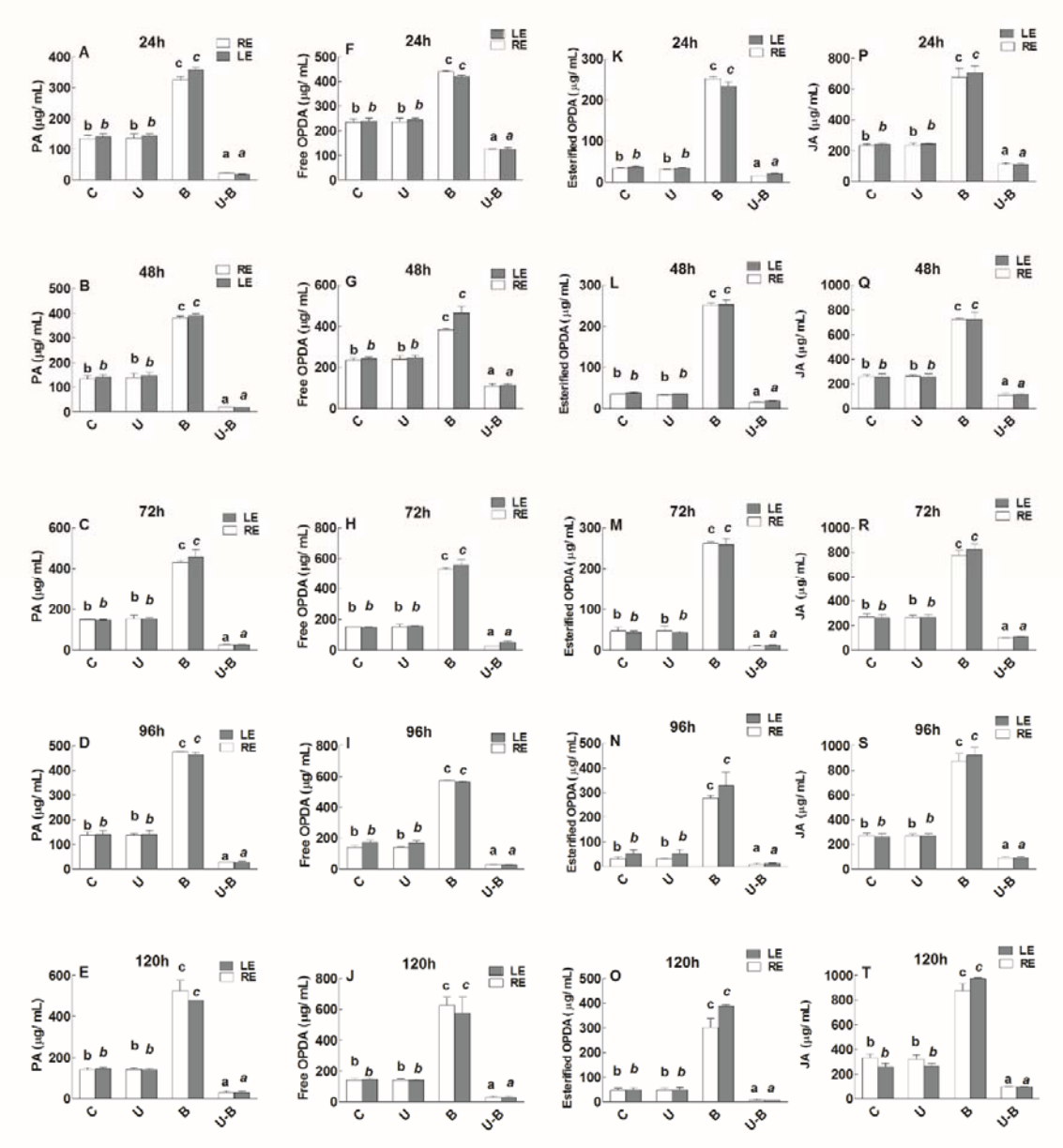
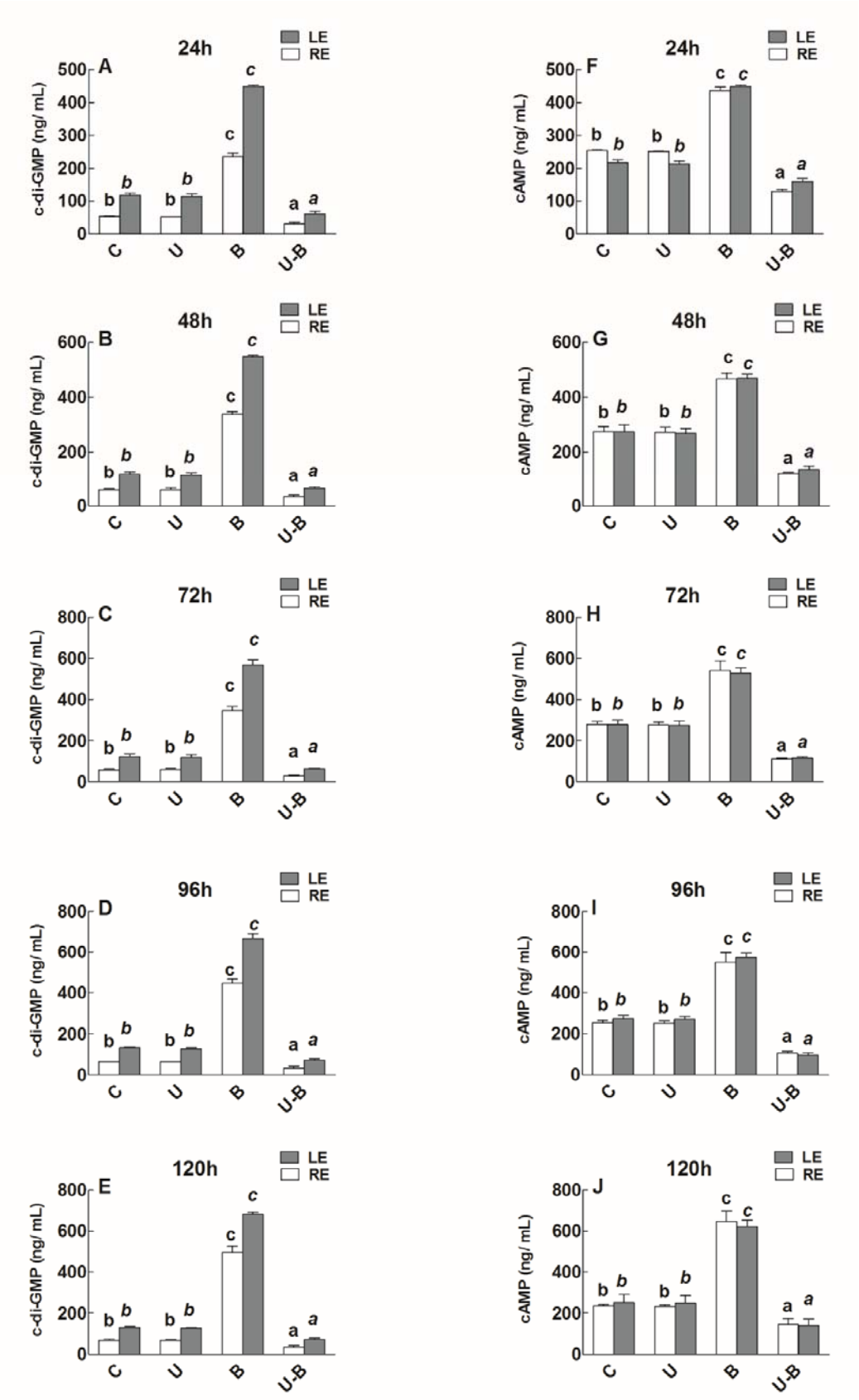


509

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 (Uniconizole), B (BIPOL), U-B (Uniconizole-BIPOL) at
514 spore density 0.6 for the different time duration. Ducan's test was performed and different
515 alphabetic letters shows the significant difference. Experiement was repeated at least three
516 time independently.



517

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 (Uniconizole), B (BIPOL), U-B (Uniconizole-BIPOL) at spore density 0.6 for
522 the different time duration. Ducan's test was performed and different alphabetic letters shows
523 the significant difference. Experiement was repeated at least three time independently.

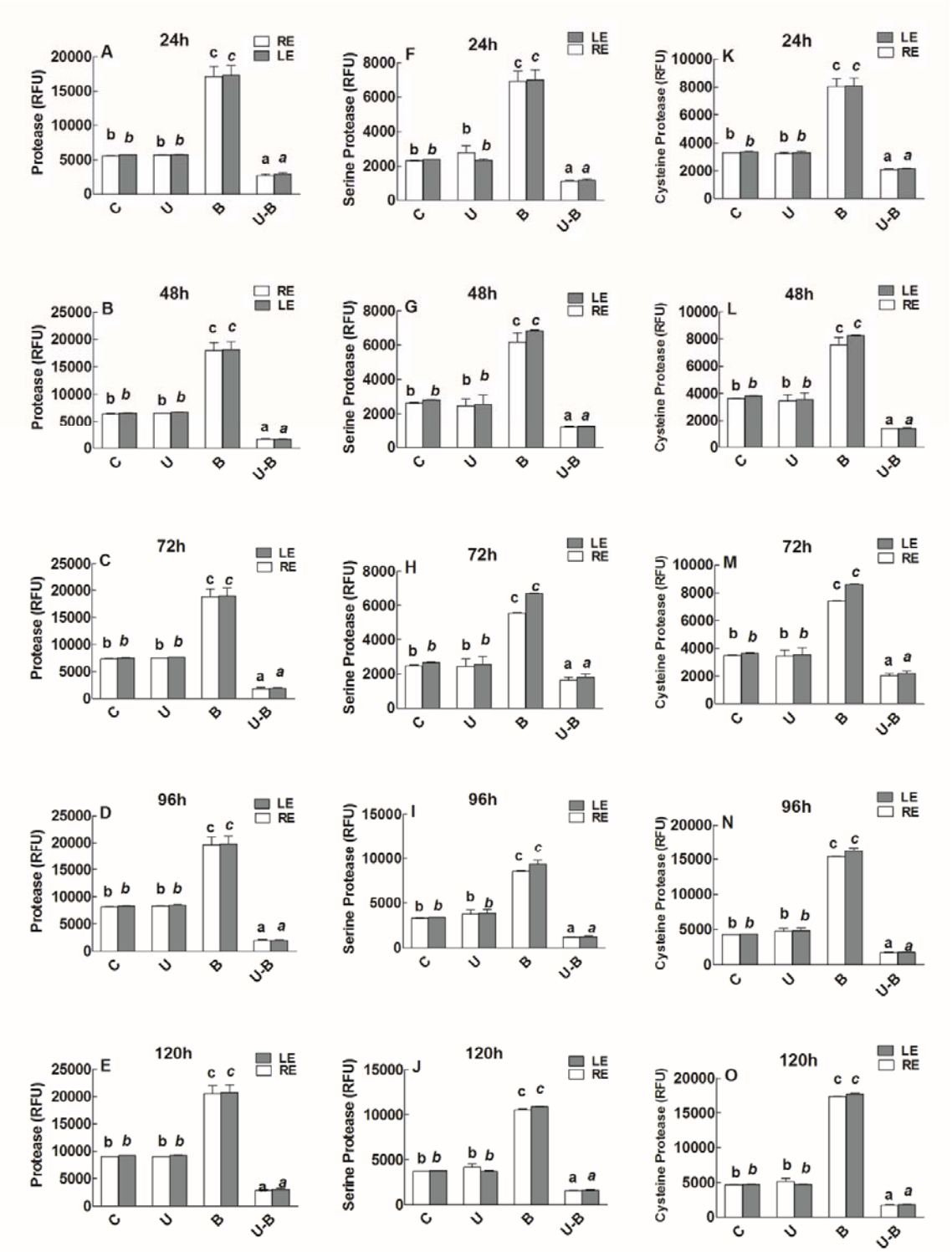


Figure 7

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 (Uniconizole), B (BIPOL), U-B (Uniconizole-BIPOL) at spore density 0.6 for
529 the different time duration. Ducan's test was performed and different alphabetic letters shows
530 the significant difference. Experiement was repeated at least three time independently.

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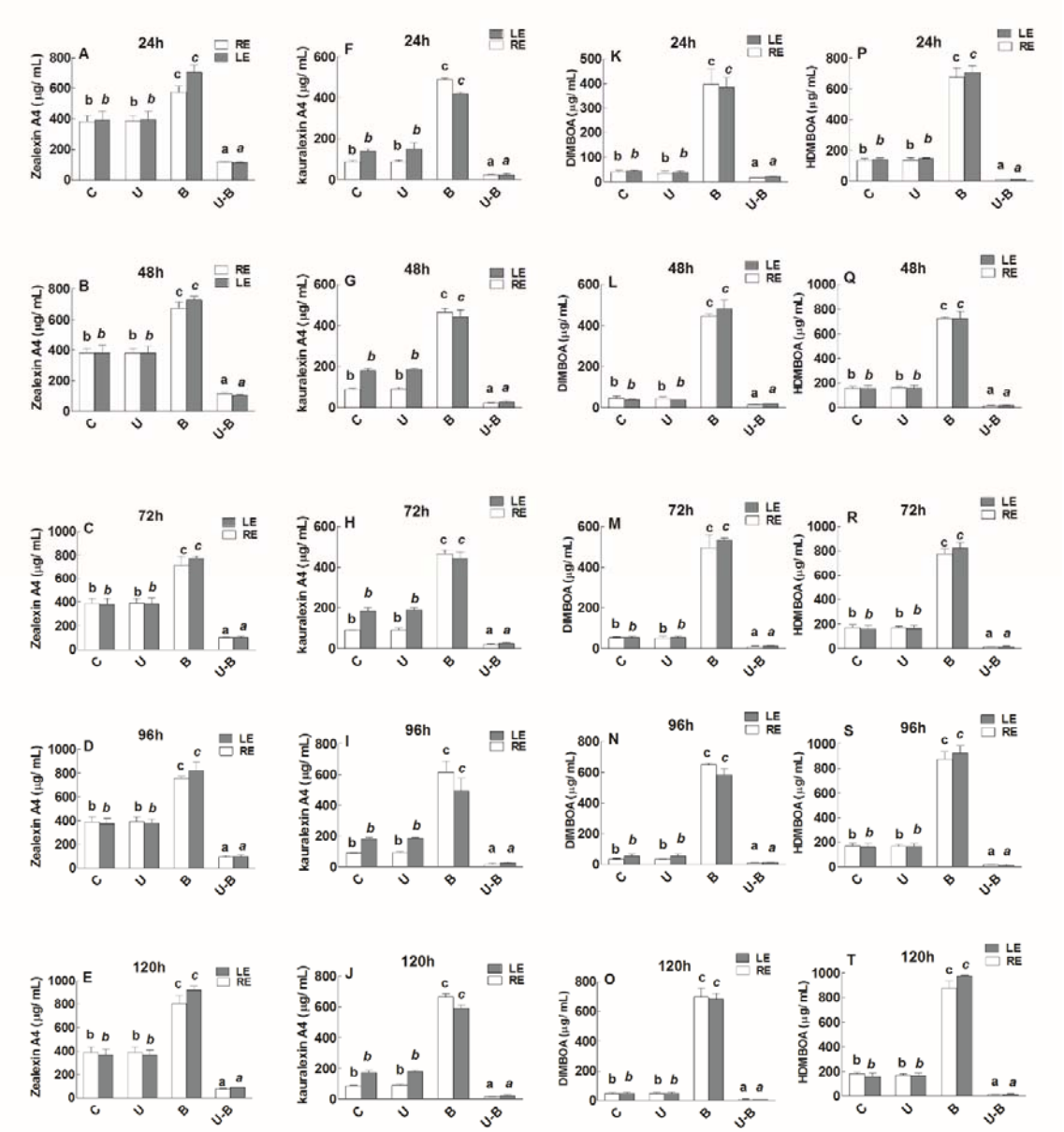

532

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 (Uniconizole), B (BIPOL), U-B (Uniconizole-BIPOL) at spore density 0.6 for the different time duration. Ducan's test was performed and different alphabetic letters shows the significant difference. Experiement was repeated at least three time independently.

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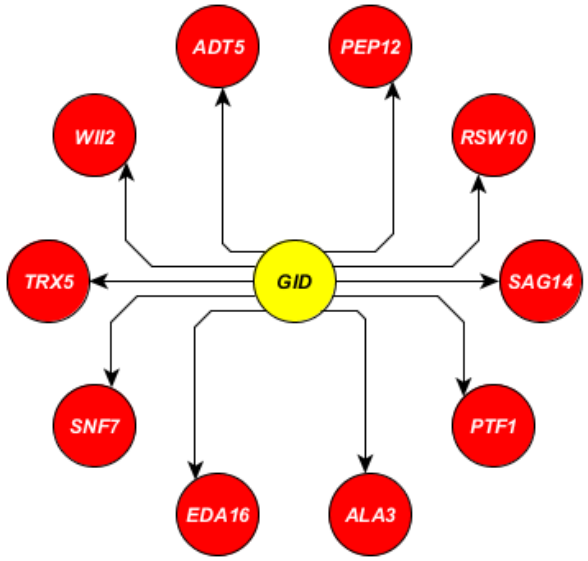

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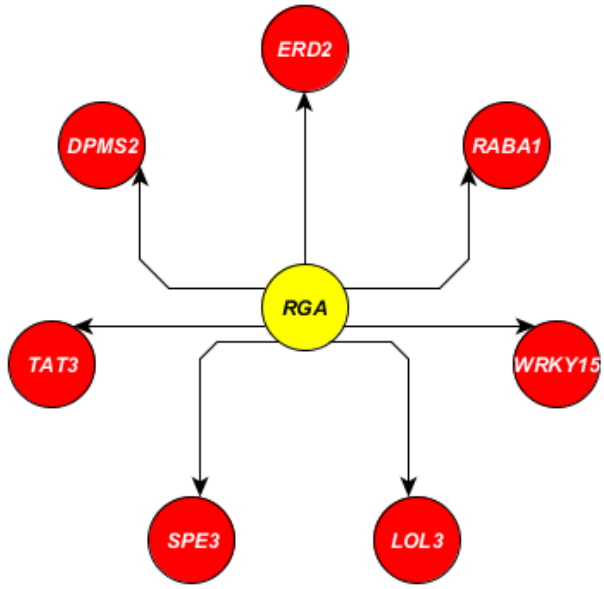

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584 **Figure 9**

585 Data extracted from Expression Angler 2016 showing the set of co-expressed genes with *GID*

586 (a) and DELLA *(Mihailova et al.)* (b)n under microbe stresses based on R- cut off range -0.7

587 to 0.9) with the principle gene expression.

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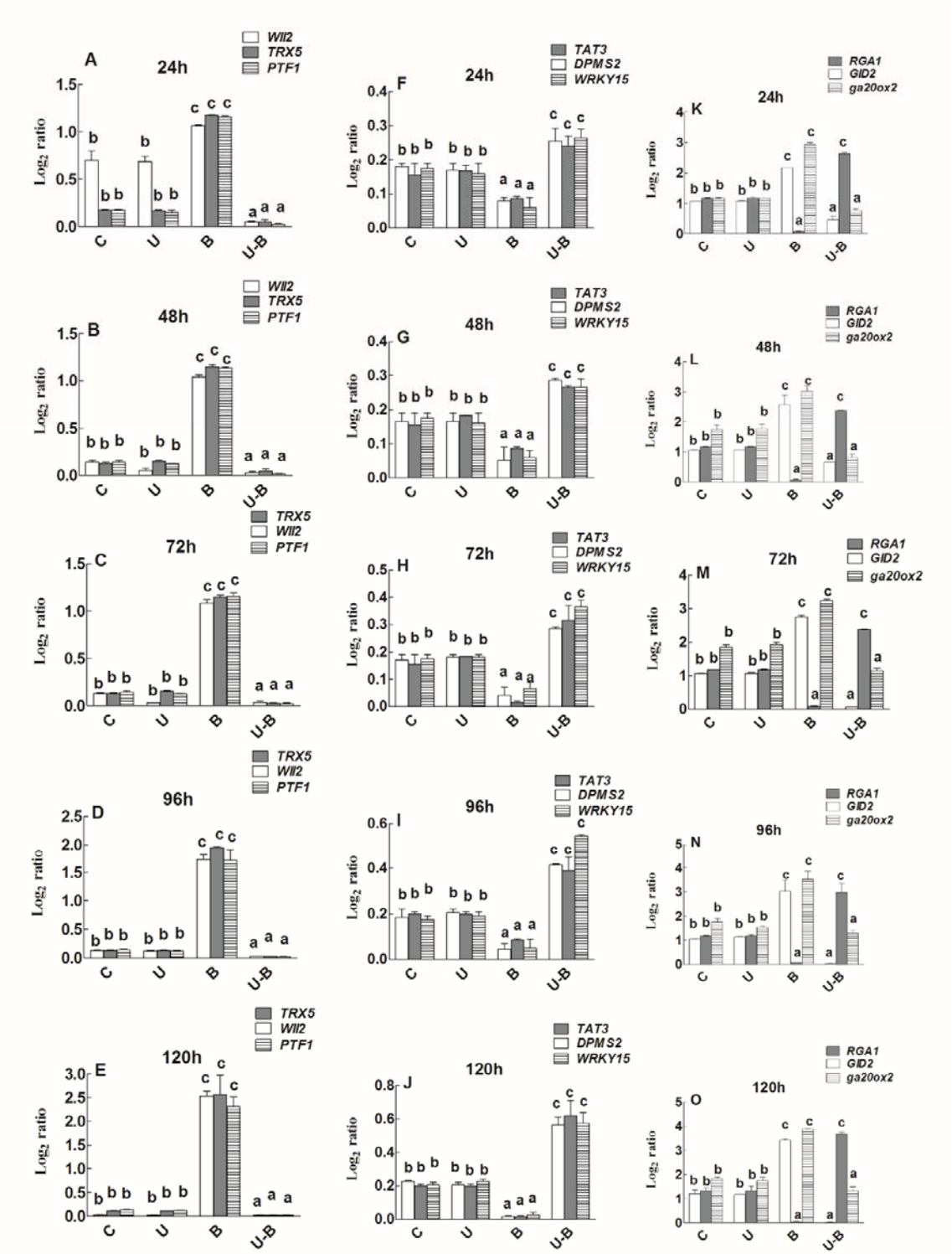


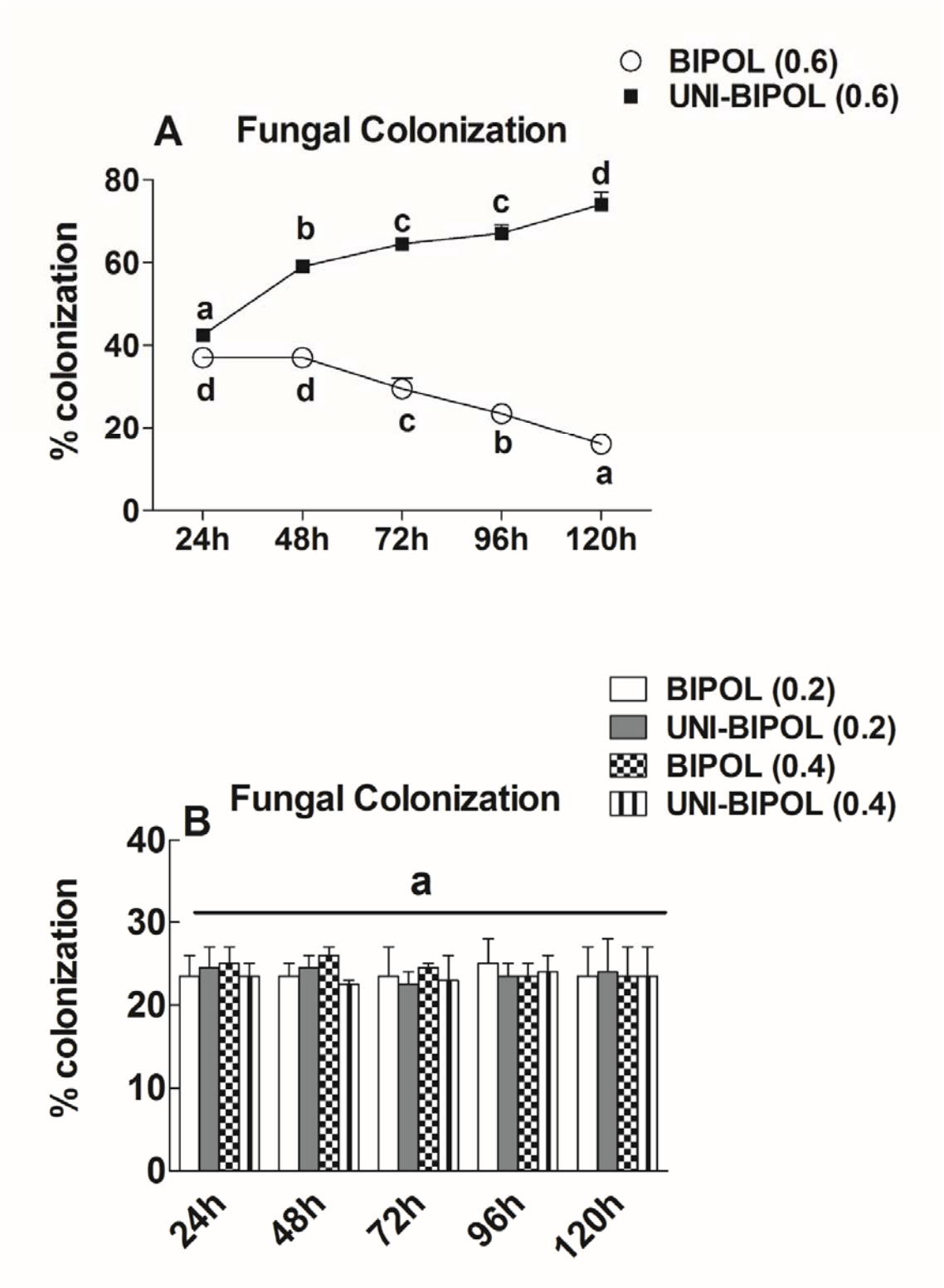
589

590 **Figure 10**

591 Determination of gene expression in the leaf tissues of 11 days old maize seedlings exposed

592 to different treatments including C (control); U (Uniconizole), B (BIPOL), U-B

(Uniconizole-BIPOL) for the different time duration. Ducan's test was performed and different alphabetic letters shows the significant difference. Experiement was repeated at least three time independently.



598 **Figure 11**

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

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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 uniconizole 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). Uniconizole 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 uniconizole treated seedlings. This clearly indicated that both SDs are not effective

649 either at pathogenic level or growth promoting level. We also examined the growth
650 suppression of Uniconizole treated seedlings and BIPOL SD ($OD_{0.6}$) treated seedling through
651 the production of secondary metabolites in host leaf and root exudates. Plant produces
652 secondary metabolites under biotic or abiotic stress to cope them at the expense of its normal
653 growth and development (Yang et al., 2018). As expected, the level of Proline, TFC (total
654 flavonoid content), PPs (phenylpropanoids) and GLs (Glucosinolates) were high in
655 Uniconizole treated seedlings and BIPOL SD ($OD_{0.6}$) treated seedling. However, after the
656 release of Uniconizole effect on the maize seedlings, the level of secondary metabolites was
657 lowered.
658 Several biotic or abiotic stresses induce oxidase activity in plants which in turn decrease
659 growth of the host plant to eliminate the stress condition (Selinski et al., 2018). As expected,
660 the treatment of uniconizole to maize seedlings increased oxidase activity and subsequently
661 decreased plant growth till 72 hours after which the oxidase activity became normal.
662 However, the oxidase activity remained high in BIPOL treatment SD ($OD_{0.6}$) with co-related
663 low growth rate of the seedlings. As opposed, high calatase activity was noted in prolifically
664 growing seedlings (Poli et al., 2018). Similarly, we observed low growth in low catalase
665 activity treatments (uniconizole till 72 hours and BIPOL SD ($OD_{0.6}$) in all duration). In the
666 contrary, BIPOL treatment SD ($OD_{0.6}$).
667 On the contrary, BIPOL SD ($OD_{0.6}$) treated seedling could not reduce the level of secondary
668 metabolites was lowered. This literally meant that stress of BIPOL SD ($OD_{0.6}$) treatment
669 remained high during the treatment hours. Additionally, the host seedlings did not respond to
670 BIPOL SD ($OD_{0.2}$ or $OD_{0.4}$) due to normal secondary metabolites concentration in both leaf
671 and root exudates. Similarly, the same treatments too did not reduce the high concentration of
672 secondary metabolites in Uniconizole treated seedlings. We determined HR in host in host by
673 quantifying the PA, pure OPDA, esterified OPDA, and JA levels in host under biotic and

674 abiotic stress (Schuman et al., 2018). As expected, the maize seedlings containing high
675 concentration of GA_3 (B seedlings treated by BIPOL SD ($OD_{0.6}$)) produced high level of HR
676 inducing molecules while the seedlings containing low GA_3 level (U-B seedlings treated by
677 BIPOL SD ($OD_{0.2}$ or $OD_{0.4}$)) produced low concentration of these molecules. However, the
678 other two SDs could not trigger the production of HR inducing molecules in neither in
679 Uniconizole treated seedlings nor control seedlings.
680 This deducted that the two BIPOL SD ($OD_{0.2}$ or $OD_{0.4}$)) treatment to maize seedlings are
681 inert, neither growth promoting and virulent. The same pattern was also followed by HR
682 signalling inducing molecules as c-di-GMP, and cAMP. In host plant under the interaction of
683 undesirable microbe, several protease activities begin which break down the proteins involves
684 in the transportation of nutrients and facilitation of microbial colonization (Havé et al., 2018).
685 We determined three marker protease activities such as universal protease activity, serine
686 protease activity and cysteine protease. The quantification of protease activities also showed
687 high HR in the maize seedling stressed with BIPOL SD ($OD_{0.6}$) only. As the HR triggered in
688 host plant due to microbe interaction, cell death inducing substances are biosynthesized
689 known as phytoalexins (Komives and Kiraly, 2019). We determined four phytoalexins
690 commonly found in maize as Zealexins A4. kauralexin A4, DIMBOA and HDMBOA (Yang
691 et al., 2019). As expected, the level these phytoalexins were high only in BIPOL SD ($OD_{0.6}$)
692 treatment.
693 Using in-silico approach, we finally extracted data regarding gene expressions in *A. thaliana*
694 under the interaction with model biotrophs and necrotroph at GA_3 hypersentivity. The
695 expression of 9 markers genes were analysed in maize seedlings using qRT-PCR. The result
696 showed that genes (*WII2*, *TRX5*, *PTF1, DPMS2, TAT3*, *WRKY15*, *GID1*, *ga20ox2* and *RGR1*)
697 inhibiting microbe colonization in host were highly upregulated in the seedlings which had
698 high GA_3 level (BIPOL treated seedlings). On the contrary, such genes were down regulated

699 in Y-B thus facilitating BIPOL interaction. The description of the genes were taken from the
700 online available database of Arabidopsis (TAIR) (Consortium et al., 2019). After overall
701 inspection, we also determined BIPOL colonization frequency on the roots of maize
702 seedlings. During plant microbe interaction, roots of the most plant host provide optimal site
703 for colonization (Hugoni et al., 2018). As expected, the BIPOL SD ($OD_{0.6}$) colonized only in
704 the roots of the uniconizole pre-treated seedlings of maize at leaf. It was because the level of
705 GA_3 was inhibited which could interfered the colonization. Due to the inhibition of GA_3, IAA
706 and Transzeatin levels were high in the seedlings. IAA (Mehmood et al., 2018) and
707 Tranzeatin (Kabbara et al., 2018) are involved to have a role in the accommodation of fungal
708 colonization on host plant roots. As opposed, in the seedling of maize where GA_3 was high
709 (control seedlings treated with BIPOL SD ($OD_{0.6}$)), the BIPOL colonization was hindered.
710 This could be due to the high level of GA_3 which developed cross talks with IAA and
711 Tranzeatin, thus decreasing its production in host.

712 **Conclusion**

713 Beside growth inducing molecules, GA_3 also indicates the compatibility of host-micorbial
714 interaction. Inoculation of BIPOL majorly caused GA_3 hypersentivity at a SD ($OD_{0.6}$) at 600
715 nm. this hypersensitivity interfered the optimal level of IAA and Trans-zeatin in the host,
716 thereby demoting growth of the seedlings. Once the GA_3 was inhibited using uniconizole
717 treatment BIPOL SD ($OD_{0.6}$) colonized on the seedlings root and resumed its growth
718 promoting acitivity. This supported our hypothesis that GA_3 hypersensitivity hinders the
719 interaction of BIPOL with *Z. mays* under its cross talks with IAA and trans-zeatin.

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Parsed Citations

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