International Journal of Current Research in
Biosciences and Plant Biology
Volume 5 • Number 9 (September-2018) • ISSN: 2349-8080 (Online)
Journal homepage: www.ijcrbp.com

Original Research Article **doi:** https://doi.org/10.20546/ijcrbp.2018.509.006

Effects of Foliar Application of 24-Epibrassinolide on Growth and Induction of Resistance of Maize Plants to Helminthosporiosis on the Field

Kuate Tueguem William Norbert[1], Ngoh Dooh Jules Patrice[2], Essono Obougou Germain Gabriel[3], Ndongo Bekolo[1], Atindo Songwe Thierry[1], Heu Alain[1], Tene Tayo Paul Martial[4] and Ambang Zachée[1]*

[1]Laboratory of Plant Pathology, Department of Plant Biology, Faculty of Science, University of Yaoundé I, BP: 812 Yaounde-Cameroon
[2]Department of Biology, Faculty of Science, University of Maroua, Cameroon
[3]Department of Microbiology, Faculty of Science, University of Yaounde I, BP: 812 Yaounde-Cameroon
[4]Department of Biochemistry, Laboratory of Phytoprotection and Plant Valorization Ressource; Biotechnology Center, University of Yaounde I, PO Box 3851 Messa-Yaounde-Cameroon

***Corresponding author.**

Article Info

Date of Acceptance:
17 August 2018

Date of Publication:
06 September 2018

Keywords

24-epibrassinolide
Helminthosporium turcicum
Induced resistance
Maize
Pathogenesis-related proteins
Secondary metabolites

ABSTRACT

This work aims to evaluate the role of 24-epibrassinolide (EBR) on the growth and resistance of two maize varieties CMS 90-15 and CMS85-01 to helminthosporiosis in natural conditions. The experimental device is a completely randomized factorial split-plot consisting of five treatments and two varieties repeated three times each. The present study was carried out during two successive seasons of 2015 and 2016 and all data are the meaning of these seasons. The agro-morphological, epidemiological and production parameters of the maize plants were evaluated under the application of the treatments used. The main results show a significant effect of EBR on plant growth, yield, disease resistance, synthesis of secondary metabolites and defense proteins (p<0.05). The EBR significantly reduced grain losses, promoting a gain of about 1.5 t/ha compared to the control and NPK treatments with 1 t/ha for the two varieties studied. Its action greatly reduced the severity with a technical efficiency of 42.3 % for the variety CMS 90-15 and 37.3% for the variety CMS 85-01. It also induced resistance of plants to helminthosporiosis, synthesis of secondary metabolites and defense proteins. These results show that 24-epibrassinolide could be used in the control of helminthosporiosis in cultivated plants.

Introduction

Maize (*Zea mays* L.) has been considered as one of the most promising cereal grains for human consumption in the world. Recently, maize has become to be the highest grain yielder in comparison to other cereals (FAO, 2015). The average global production of maize in 2013 was 1016740 tons as compared to 745,710 tons of rice and 713,183 tons of wheat and other crops (FAO, 2015). It is one of the most widely grown cereals in the world (Charrier et al., 1997) and of major economic importance, is a raw material in the brewing, cosmetics, energy and animal industries and basic food for human. It is eaten fresh, roasted, or as boiled corn and in the form of flour to produce puff-puff, pap or corn fufu (Okoruwa, 1995).

In Cameroon, 90 % of maize grown mainly by peasants (1,029,000 tons) does not meet national demand of 1,106,000 tons. In addition to technical difficulties, one of the main causes of low yields is the vulnerability of plants to diseases such as helminthosporiosis which is present in all agro-ecological zones (Ngoko, 1994). Faced with this situation, in several research pools and research institutions such as the Agricultural Research Institute for Development (IRAD), many efforts have been put in place to improve maize production which has led to the creation of several resistant or early hybrid composites varieties. However, none of these varieties, cannot grow rapidly, produce high yields and resist various stresses at the same time. These parameters are controlled by the massive use of plant protection products. In recent years, the systematic use of chemicals has been increasingly challenged for its negative impacts on the environment and consumers' health.

Generally, environmental problems have caused a great change in plant protection strategies. This has led to the removal of 750 active substances from the 1,000 molecules which were placed on the European market before 1993 and which have been re-evaluated by the EFSA (European Food Safety Authority). Reduction in the number of active products has led to development of alternative methods for the fight against plants parasites (El Guilli et al., 2009). One of these strategies is the optimization of the natural defense mechanisms of plants during plant-parasite interaction. It also involves the stimulation of natural defenses by elicitors or stimulators of plants. Thus, the purpose of this work is to study the effects of 24-epibrassinolide in the resistance mechanisms of maize plants to helminthosporiosis caused by *Helminthosporium turcicum*.

Materials and methods

Materials

A) The plant material consists of the seeds of two varieties of maize, whose characteristics are grouped in Table 1.

B) 24-epibrassinolide (EBR) with the following structure (Fig. 1), Michael et al., (1979), being the most efficient brassinosteroid (BR) and the only one currently being used in agriculture and agro- Chemistry (Krishna, 2003) is used in this work;

C) Agricultural inputs and fertilizers such as droppings from fowls, ridomil Gold Plus, Chloromethyl pyrufox, fertilizers (NPK 20-10-10) are used.

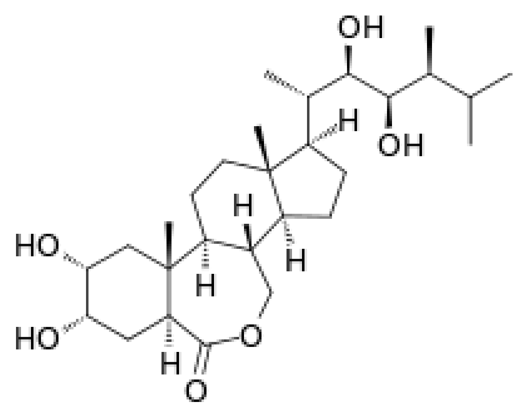

Fig. 1: structure of 24-épibrassinolide (Michael et al., 1979).

Table 1. Characteristics of the seeds used (CMS: Cameroon Maize Selection).

Varieties	Cycles (days)	Colors	Origin	Helminthosporiosis sensitivity
CMS 85-01	85	White	IRAD Nkolbisson	Weakly
CMS 95-01	90-95	White	IRAD Maroua	Average

Methods

The experimental site, on the peasant farm was marked out, cleared and then tilled in the central region of Cameroon. This work was carried out on a multifactorial split-plot device with three completely randomized identical blocks consisting of five treatments (main factor) and two varieties (secondary factor) with three repetitions each during two successive seasons (years: 2015 and 2016). The treatments are; T0: control with tap water; T1: 24-epibrassinolide; T2: ridomil gold plus; T3: NPK fertilizers; T4: 24-epibrassinolide + fertilizer (NPK) + ridomil. Plots of 3 m x 15.5 m dimensions each were separated by 1 m. Within each plot, four lines of maize (two lines for each variety) were separated from each other by a space of 0.50 m. The pots of two seeds are 0.70 m apart. Before sowing, each variety was divided into two batches, one soaked in sterile distilled water and the other in a 24-epibrassinolide (EBR) concentrated solution of 0.001 ml per ml for 24 hrs.

All treatments including treatment with EBR were then applied from the 26th day after sowing (DAS) and their frequencies depend on the recommendations of the experimentalist. To avoid any competition, the plots were cleared. EBR and Ridomil where applied by spray while NPK where applied on grown around the plants. Growth parameters evaluated in the field are; rate of growth after sowing; number of leaves appearing on the plants in the various treatments from the 10th DAS until flowering; height of the stem and yield which is calculated as follows.

$$Rdt = \frac{PTxNPt}{nPtT}$$

Where, Rdt = yield per hectare, PT = weight per treatment, NPt = average number of seedlings per hectare and nPtT = number of plants per treatment.

Epidemiological parameters were also evaluated. The assessment of the disease consisted of identifying the symptoms of helminthosporiosis naturally occurring in farm. This was followed by the evaluation of disease incidence every 10 days from the 41st DAS using the following formula.

$$I = \frac{n \times 100}{N}$$

Where, I: is the disease incidence, n is the number of diseased plants per plot and N the total number of plants sampled per plot.

The degree of infection or severity of the disease is evaluated by estimating the leaf area occupied by disease symptoms using the following formula; $S = \Sigma\ (ab)\ /\ N$, Where Σ (ab): is the sum of the products of the number of diseased plants (a) by the degree of infection (b) given in % and N: is the number of diseased plants (Tchoumakov and Zaharova, 1990). The technical efficiency of the products used is calculated using the following formula.

$$E = \frac{Sc - Str}{Sc} x100$$

Where, E: is the technical efficiency, Sc is the severity of the control and Str is the severity of the treated plot.

Extraction and determination of secondary metabolites and defense proteins

It consists of extraction and determination of secondary metabolites and defense proteins found in the leaves (maize plants) collected from the farm at the beginning of flowering 7 days after application of treatments to avoid the direct influence of the latter on the mechanisms of resistance of the plants.

Extraction and dosage of soluble and parietal phenols

The extraction steps are summarized as shown below Fig. 2. The determination of total soluble phenolic compounds in the extracts obtained is done according to the method described by Marigo (1973). A spectrophotometer (UV-1605, UV-visible SPECTROPHOTOMETER SHIMADZU) is used to measure the absorbance at 760 nm.

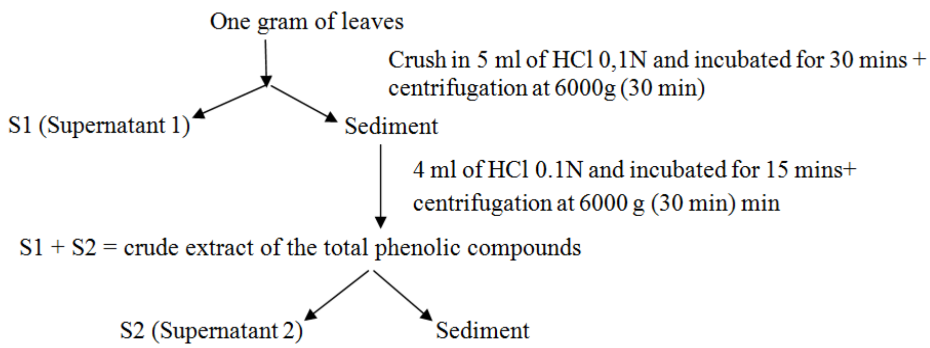

Fig. 2: Extraction steps of total phenolic compounds.

Extraction soluble and parietal proteins

One gram of leaves of each variety is mixed with 5 ml of sodium acetate buffer (0.4 mol / l, pH 5), 1 g of sand, and then crushed in a mortar. The extract is centrifuged for 20 minutes at 5000 rpm and at 4°C. The supernatant is used to determine soluble and parietal proteins.

Determination of soluble and parietal proteins

Determination of total soluble proteins obtained from the extracts is done using Bradford's method (1976). This colorimetric method determines the quantities of proteins contained in a solution using a reagent which contains Coomassie G250 blue. In the presence of proteins, the light brown Coomassie blue reacts with the hydrophobic residues of the amino acids constituting the protein. The solution then turns blue, thereby displacing the maximum absorption peak from 465 nm to 595 nm.

Measurement of peroxidasic activity of soluble and ionically linked proteins

Peroxidasic activity (POX: EC 1.11.1.7) in proteins is carried out according to the method described by Baaziz et al. (1994) by estimating the initial rate of the enzymatic reaction. The substrate used is guaiacol which is oxidized in the presence of H_2O_2 to tetragaiacol by peroxidases as shown in the equation below:

$$\underset{\text{Gaïacol}}{4\,(C_7H8O_2)} + 4H_2O_2 \xrightarrow{\text{POX}} \underset{\text{Tetragaïacol}}{(C_7H_8O_2)_4O_4} + 8H_2O$$

A spectrophotometry at 470 nm is used to examine the appearance of tetragaïacol which is expressed in ΔA470/min/g of fresh material

Measurement of polyphenoloxidase (PPO) enzyme activity

The determination of polyphenoloxidase activity (PPO: EC 1.10.3.1) is carried out using the methodology described by Van Kemmenn and Broumer (1964) using catechin as substrate. The optical density is read at 330 nm and the PPO activity is expressed as Δ330/min/g of fresh material.

Measurement of the enzymatic activity of glucanases

The enzymatic activity of the glucanases is carried out following the modified method of Pirovani et al. (2008). A spectrophotometer (UV-1605, UV-visible SPECTROPHOTOMETER SHIMADZU) at 540 nm is used to measure the absorbance.

Statistical analysis

The results obtained are analyzed with the SPSS 16.0 software. The significance difference between the treatments is evaluated by the Duncan test at 5 % threshold. When the differences are significant, the multiple comparison tests using Duncan's ANOVA is used.

Results

Growth parameters

Growth rate

After sowing, growth rate of plants in the farm showed a significant effect on the varieties, especially those treated with ridomil. Plots treated with ridomil showed the lowest growth rate. However, it is observed that the variety CMS 8501 had the best growth rate of 96.4 ± 1.09 % compared to 93.1 ± 1.6 % for variety CMS 9501(Fig. 3). These values are the meaning of data obtained during two successive seasons (2015 and 2016).

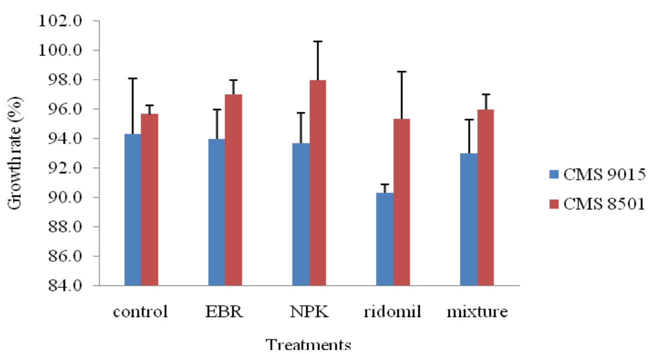

Fig. 3: Growth rate of maize seeds after treatment with EBR. Values are means of 2 replicates.

Evolution of the number of leaves

At the 14th DAS the number of leaves showed no significant difference between treatments. At the 33rd DAS (Fig. 4a), the treatments applied had a significant effect on the increase in the number of leaves. These values are the meaning of data obtained during two successive seasons (2015 and 2016).

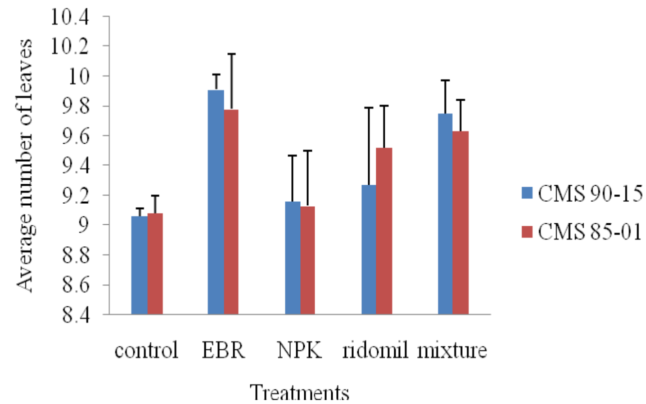

Fig. 4a: Effect of EBR on the average number of leaves produced at 33rd DAS. Values are means of 2 replicates.

Evolution of the height of the stems

The treatments show a significant effect on the increase in height of the stems, especially those with EBR and a mixture of the treatments for the two varieties (Fig. 4b). These values are the meaning of data obtained during two successive seasons (2015 and 2016).

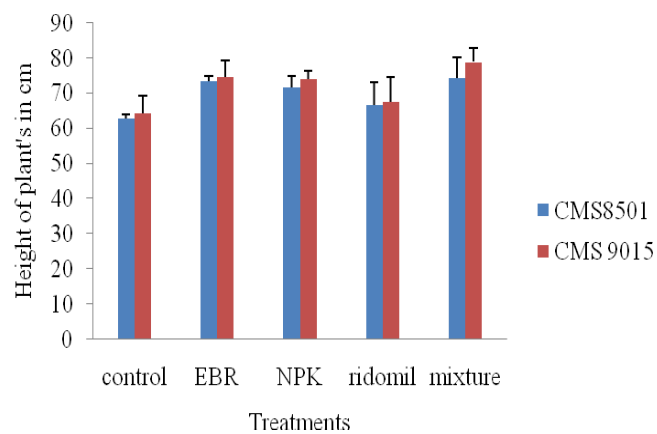

Fig. 4b: Evolution of plants' height after treatment at the 33rd DAS. Values are means of 2 replicates.

Evolution of flowering

The results recorded on the table below show the significant effect of EBR on the evolution of flowering. From the 50th DAS the highest flowering rates are recorded in the EBR and Mixture treatments Table 2. Data are the meaning obtained during two successive seasons (seasons: 2015 and seasons 2016).

Table 2. Evolution flowering with time.

Treatments	Varieties	50^{th} DAS	53^{th} DAS	57^{th} DAS	61^{th} DAS
Control	CMS 90-15	4.33±2.51	29.67±10.21	88.66±3.21	99.07a
	CMS 85-01	3.00±1.00	38.00±5.00	87.80±1.21	100a
EBR	CMS 90-15	10.00±2,00	67.00±8.71	93.00±1.73	100a
	CMS 85-01	7.33±2.51	57.67±3.05	95.88±0.8	100a
NPK	CMS 90-15	7.00±1.00	63.33±4.93	92.55±0.8	100a
	CMS 85-01	7.33±0.57	58.33±3.51	91.13±2.80	100a
Ridomil	CMS 90-15	3.33±1.52	30.00±7.93	83.43±5.87	100a
	CMS 85-01	3.33±0.57	32.00±3.00	84.66±2.88	99.33a
Mixture	CMS 90-15	10.00±4.00	69.33±12.22	95.33±5.05	100a
	CMS 85-01	7.33±3.21	70.33±12.66	96.45±5.80	100a

* Data with the same letters in the same column are not statistically different at a 5% threshold. Values are means of 2 replicates.

Epidemiological parameters

Incidence of helminthosporiosis on maize plants on the farm

During two successive seasons (2015 and 2016), the data recorded on the incidence of helminthosporiosis on the field (Fig. 5a) show that before flowering (50^{th} DAS), more than half of the plants are infected in all treatments. This incidence varies between 80% for CMS 9501 of the control and 53.3% for CMS 8501 of treatment M. Subsequently, the incidence of disease reached 100 % in all the treatments despite multiple applications with antifungal treatments (Fig. 5b).

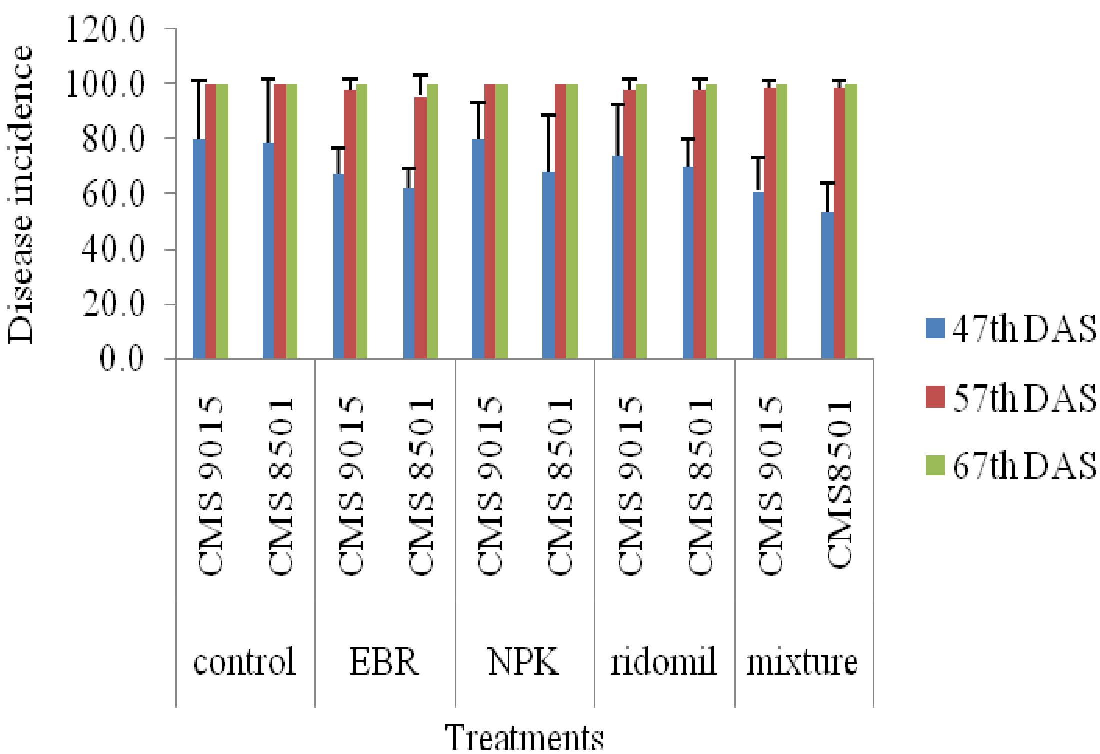

Fig.5a: Evolution of helminthosporiosis with time. Values are means of 2 replicates.

Fig. 5b: Helminthosporiosis degree attack. A: Plant treated with EBR and B: Control.

Severity of helminthosporiosis on maize plants in the field

Despite the high incidence of helminthosporiosis in all treatments, the severity of the disease remains low in the studied plot. At the 47th DAS less than 1 % of disease severity is recorded in all treatments. The 77th DAS show a rapid increase in the severity rate in all treatments. However, the lowest rates are obtained in the plots treated with EBR and Rd. The evolution of the disease depends on the treatments. It is greatly reduced in EBR (14.6 and 15.64 %), Rd (10.9 and 10.36 %) and M treatments (11.34 and11.99 %), respectively on plants of varieties CMS 90-15 and CMS 85-01 (Table 3). There was no significant difference between maize varieties in each treatment. Technical efficiency is high in these three last treatments.

Table 3. Evolution of disease severity on maize plants in the field.

Treatments	Varieties	47th DAS	57th DAS	67th DAS	77th DAS	Technical efficiency
Control	CMS 90-15	0.8±0.2a	1.72±0.15a	12.2±1.38a	25.29±2.10a	-
	CMS 85-01	0.8±0.2a	1.57±0.34a	12.82±1.20a	24.95±1.85a	-
EBR	CMS 90-15	0.2±0.2b	0.98±0.10b	8.6±1.85bc	14.6±0.97c	42.30
	CMS 85-01	0.1±0,1b	0.98±0.16b	8.12±0.37bc	15.64±1.14c	37.31
NPK	CMS 90-15	0.62±0.12a	1.51±0.22a	11.11±3.22ab	20.41±2.11b	19.30
	CMS 85-01	0.56±0.06a	1.49±0.36a	11.11±2.26ab	20.83±0.74b	16.51
Ridomil	CMS 90-15	0.73±0.15a	0.65±0.18b	6.61±0.97c	10.94±1.36d	56.74
	CMS 85-01	0.74±0.3a	0.58±0.16b	6.15±0.65c	10.36±0.95d	57.70
Mixture	CMS 90-15	0.22±0.2b	0.63±0.15b	6.5±1.41c	10.67±0.45d	57.80
	CMS 85-01	0.13±0.06b	0.66±0.20b	6.73±1.53c	10.99±0.71d	55.20

* Data with the same letters in the same column are not statistically different at a 5 % threshold. Values are means of 2 replicates.

Biochemical parameters

Quantity of soluble and parietal phenols

Treatments in the field show a significant effect on the quantity of total phenolic compounds. These quantities are influenced by the application of 24-epibrassinolide compared to control. The largest quantities are recorded in 24-epibrassinolide and fertilizer treatments (Fig. 6). It appears from this figure that the variety CMS 85-01 produces more phenolic compounds than CMS 90-15 variety with 9.88 and 6.91 GAEq/mg PMF respectively for EBR.

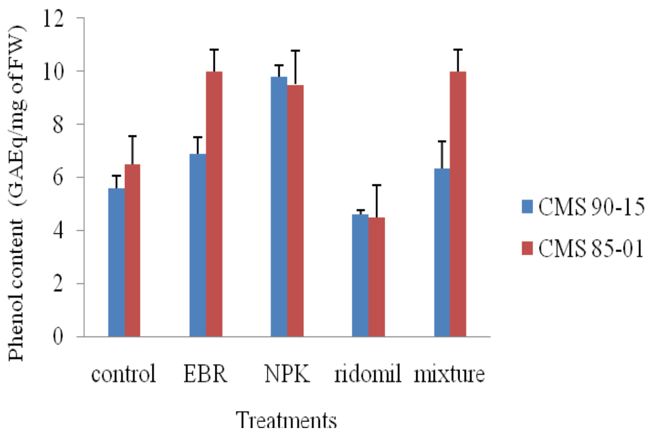

Fig. 6: Effect of EBR on the quantities of phenol in Gallic Acid Equivalent / mg of FW. Values are means of 2 replicates.

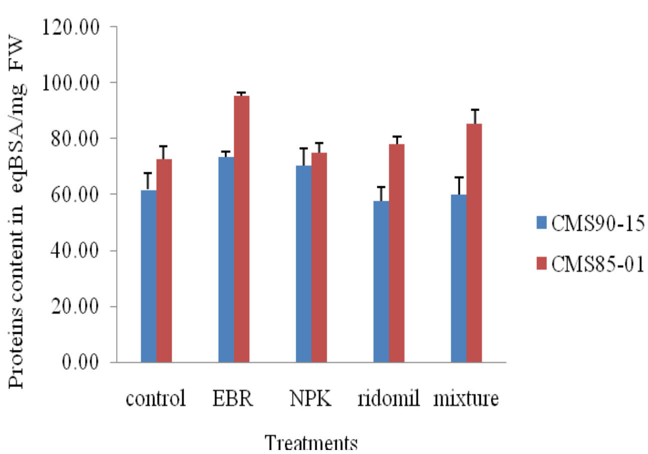

Fig. 7: Effect of EBR on the content of soluble and total proteins. Values are means of 2 replicates.

Content of soluble and parietal proteins

The content of soluble and parietal proteins is influenced by EBR 73.4 ± 2.03 for CMS 90-15 and 95.20 ± 1.32 for CMS 85-01. Generally, the content of soluble and parietal protein is influenced by the treatments and the varieties. The best values are obtained in the variety CMS 90-15 (Fig. 7).

Measurement of enzyme activity of POX, PPO and PR2

The enzymatic activity of β-1,3-glucanase (PR2) proteins shows a significant difference between treatments (Table 4). This ability of proteins to break down the substrate is prominent with 24-epibrassinolide treatment. Generally, it is observed that EBR stimulated the synthesis of PR2 proteins in the two varieties. A significant difference was observed in the enzymatic activity of POX (POX: EC 1.11.1.7) and PPO (PPO: EC 1.10.3.1) (p <0.05), particularly for leaves treated with 24-epibrassinolide. For the two varieties studied, enzymatic activity is not the same. There is a strong activity of peroxidases and PR2 for variety CMS 85-01 with respect to CMS 90-15 variety, implying a greater presence of these enzymes in CMS 85-01.

Grain yield

The yield of grains at harvest is influenced by plant protection products (Table 5). Yields recorded with EBR, NPK and M treatments for CMS90-15 and CMS 85-01 varieties are: 4.02± 1.13 - 3.84 ± 1.03 t/ha; 3.31 ± 0.51 - 3 ±0.39 t/ha and 3.85 ± 0.44 - 3.04 ± 0.14 t/ha respectively. The lowest yields are recorded in the control and ridomil treatments.

Discussion

This work is based on the optimization of the defense mechanisms of maize plants in the presence of 24-epibrassinolide. There is no effect on growth rate of plants whose seeds were treated with 24-epibrassinolide before sowing. This may be caused by a very short growth period and or the non-activation of the biochemical mechanisms of grains.

Table 4. Measurement of enzyme activity of POX, PPO and glucanase (PR2).

Treatments	POX Activity ΔA470/min/g MF		PPO Activity ΔA330/min/g MF		PR2 Activity µMol glucose eq.min^{-1}	
	CMS 90-15	CMS 85-01	CMS 90-15	CMS 85-01	CMS 90-15	CMS 85-01
Control	0.64cd	0.80bcd	0.077e	0.11de	9.61g	42.6b
EBR	1.03a	1.08a	0.33ab	0.20cd	25d	57.4a
NPK	0.54cd	0.91ab	0.27bc	0.13de	14.8f	35.4c
Ridomil	0.45d	0.64bcd	0.096de	0.093de	18.87ef	35.9c
Mixture	0.87abc	0.86abc	0.28bc	0.38a	20.7de	44.8b

POX: Peroxidase; PPO: Polyphenoloxidase; PR2: pathogenesis-related proteins. * Data with the same letters in the same compound are not statistically different at a 5% threshold. Values are means of 2 replicates.

Table 5. Grain yields for CMS 90-15 and CMS 85-01 with different treatments in tons per hectare.

Varieties	Yield in tons per hectare	
Treatments	CMS 90-15	CMS 85-01
Control	2.56±0.42 c	2.38±0.3 c
EBR	3.86±0.39 a	3.74±0.3 a
NPK	3.95±0.31 a	3.60±0.83 ab
Rd	2.73±0.70 bc	2.51±0.20 c
Mixture	4.10±0.175 a	3.7±0.23 a

* Data with the same letters in the same column are not statistically different at a 5% threshold; Values are means of 2 replicates.

Results obtained for ridomil treatment would have been influenced by biotic agents which digested or dug up the seeds before germination. Subsequently, plants treated with EBR and NPK fertilizers are well developed in terms of growth and yield compared to those in the control and those treated with ridomil (p<0.05), confirming the growth-promoting properties of 24-epibrassinolide as shown By Kim and Wang (2010) and Michael et al. (1979) after treating soybean sprouts with pollen extracts from rape plant, Steven (1998). NPK which is the only source of nutrients to plants promotes their growth leaving them vulnerable to pathogenic attack. This result to loss in quantity and quality of grains and feed as shown by a previous study carried out by this research team. Treatment of the plants with systemic fungicides reduced the incidence and severity of the disease resulting to an increase in dry weight which led to poor growth and low yield, explaining low grain yield of 2.51 ± 0.20 t / ha and 2.73 ± 0.70 t / ha for CMS 85-01 and CMS 90-15 respectively. This is with respect to the results obtained by Siegfried et al. (2007). Treatment of plants with 24-epibrassinolide give them a high rate of productivity and resistance to diseases. This result confirmed those obtained by Dong et al. (2013) and Mohammed and Sarah (2016). The increase in growth parameters in the presence of 24-epibrassinolide increased the rate of photosynthesis. These results confirm those obtained by Zulkarami et al. (2014) and Yu et al. (2004). The effective action of EBR would be due to the complexity of its molecule as demonstrated by Zhu et al. (2012), or its potential ability to induce defense molecules such as phenolic compounds, proteins thus confirming the work carried out by Nakashita et al. (2003) on tobacco and rice. The high incidence of helminthosporiosis in the experimental plots is due to the high concentration of its pathogen in the area of study, confirming the work of Ngoko, (1994) which shows the presence of *H. turcicum* in all agro-ecological zones in Cameroon. Treatment with EBR had a significant effect on the evolution of *H. turcicum* severity. This low severity compared to the control would be due to the eliciting power of EBR which acts on certain genes causing the synthesis of the molecules and defense proteins. This confirms those obtained by Nakashita et al. (2003). Concerning the effectiveness of the products, EBR resulted to yield gains of about 1.5 t / ha relative to the control (58 %). The technical efficiency of the EBR is 39.8 % on average compare to 57.2% for ridomil gold plus. Other studies on resistance and productivity of wheat in the field in the presence of EBR (5 mg/ha) subjected to attack by *Helminthosporium sativum* and *Erysiphe graminis* showed a slight decrease in the disease between 5 – 10%, with a 20% increase in productivity (Khripach, 1999). In this study, the effect of EBR on the growth of the two varieties and the induction of resistance to *H. turcicum* via the stimulation of their intrinsic defense has resulted to a significant gain of 58 % in yield compare to 20% recorded in the previous work. This could be explained by the doses used or the surrounding microclimate. On the other hand, the treatment of seeds before sowing and the preventive and curative treatment with EBR during the vegetative phase had a positive impact in the control of fungal infection and this confirms the work carried out by Ambang (1996).

The high phenol content in the leaves of plants treated with EBR compared to the control shows its eliciting character. Nakashita et al. (2003), showed that the resistance of tobacco to Tabacco Mosaic Virus (TMV) after treatment with Brassinosteroid (BR) is due to the biosynthesis of campesterol, a precursor to brassinolides which acts on the intrinsic resistance genes of plants and triggers their transcription. The presence of EBR influences the content of soluble and parietal proteins compared to the control, 73.4 ± 2.03; 67±6.0 for CMS 90-15 and 95.20 ± 1.32; 72.7±4.52 for CMS 85-01 respectively.

The enzymatic activity of polyphenoloxidases (PPO) obtained in this study have shown that EBR stimulated the synthesis of polyphenoloxidase in maize leaves after treatment, which explains the low severity rates recorded in the EBR and Mixture for the two varieties compared to the control. These results confirm those obtained by Kim et al. (2001) after application of salicylic acid on cassava leaves and agree with Ngadze et al. (2012) observations who reported that tolerant genotype accumulates high amount of biochemical substances such as total polyphenol, POX, PPO. Many literatures have shown that PPO would intervene in the defense of plants against pathogens in many ways. By their proteolytic activity, they accelerate the apoptosis of the cells and thus limit the progression of the pathogens in the infected organs, Kuwabara and Katoh (1999). In addition, some phenols such as scopoletin and esculetin become toxic to fungi when oxidized by PPO (Gümez Vásquez et al., 2004). This role of PPO in plants to fight against pathogens was confirmed by Wititsuwannakul et al. (2002), which demonstrated a high activity of these enzymes in the GTI clone of rubber tree which has a high resistance to diseases. Enzymatic activity of glucanases (PR2) and peroxydases is very high in plants treated with EBR compared to the control. This result could be due to the following reactions: a direct response induced by the pathogen itself and an indirect response due to the eliciting effects of brassinosteroids. These results showing an increase in POX and PPO activities when leaves are treated with EBR correspond with those obtained by Navodit and Prabir (2014) and Mohammed and Sarah (2016). In the previous studies, many authors have shown the interaction of PPO and POX in the resistance of plants to diseases (Ondobo et al., 2017; Effa et al., 2015). These enzymes are involved in the defense mechanisms of virulent or avirulent pathogens, Housti et al. (2002), Constabel et al. (2000) and Kim et al. (2010). Similarly, the activity of glucanases was shown to be greater in EBR-based treatments than in controls. Generally speaking, EBR stimulated the enzymatic activity of β-1,3 glucanases in the varieties used. This result would be due to the elicitory character of brassinosteroids and confirms those obtained by Zhu et al. (1994). Plant treated with 24-epebrassinolid shown better resistance to helminthospiosis and high level PR2 activity According to Vaiyapuri et al. (2012), who demonstrated that, β-1,3-glucanases are directly involved in defence by hydrolysing the cell walls of fungal pathogens.

Conclusion

Corn is a major economic commodity in the world. Generally, its production remains low in developing countries and in Cameroon in particular because of numerous attacks caused by pathogens, especially *Helminthosporium turcicum*. The latter is responsible for corn helminthosporiosis or leaf burns which can cause losses of 20 to 90% in yield in the absence of phytosanitary treatments. The results obtained in this work show that 24-epibrassinolide compared to the control, does not only provided a vegetative growth, a good yield but also a better resistance to the disease through the stimulation and synthesis of the defense substances. Because of these potentials, EBR can be used in large scale cultivation of maize for commercial purposes.

Conflict of interest statement

Authors declare that they have no conflict of interest.

Acknowledgement

To people who contributed to the success of this work especially teachers, students and technicians.

References

Ambang, Z., 1996. Efficacité des nouveaux pesticides contre le mildiou et le doryphore de la pomme de terre, leur action et migration dans les tissus de la plante et dans le sol. Thèse de doctorat Ph.D. Université de Russie de l'Amitié des Peuples. 138p.

Baaziz, M., Aissam, F., Brake, Z., Bendiap, K., El Hadrami, I., Cheick, K., 1994. Electrophoretic patterns of acid soluble proteins and active isoformes of peroxidase and polyphenoloxidase typifying calli and somatic embryos of two reputed date palm cultivar in Morocco. Euphytica. 76, 159-168.

Bradford, M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principles of protein dye binding. Anal. Chem. 72, 248-254.

Charrier, A., Jaquot, M., Hamon, S., Dominique, N., 1997. L'amélioration des plantes tropicales. CIRAD et ORSTOM, Paris. 623p.

Constabel, C. P., Lynn, Y., Patton, J. J., Christopher, M. E., 2000. Polyphenol oxidase from hybrid poplar. Cloning and expression in response to wounding and herbivory. Plant Physiol. 124, 285-295.

Dong-Lei, Y., Yinong, Y., Zuhua, H., 2013. Roles of plant hormones and their interplay in rice immunity. Mol. Plant. 6(3), 675–685.

Effa, O. P., Niemenak, N., Djocgoue, P. F., Ondobo, M. L., Omokolo, N. D., 2015. Heritability of polyphenols, anthocyanins and antioxidant capacity of Cameroonian cocoa (*Theobroma cacao* L.) beans. Afr. J. Biotechnol. 14(36), 2672-2682.

El Guilli, M., Achbani, E., Fahad, K., Jijakli, H., 2009. Biopesticides: Alternatives à la lutte chimique? In : Agriculture durable en region méditerranéenne (AGDUMED) ; Rabat, Maroc. pp.266-280.

FAO, 2015. FAO Statistical Pocket book 2015, World Food and Agriculture Organization of the United Nations. Published by FAO, Rome, Italy, 28p.

Gümez-Vásquez, R., Day, R., Buschmann, H., Randles, S., Beeching, J. R., Cooper, R., 2004. Phenylpropanoids, phenylalanine ammonia-lyase and peroxidases in elicitpr-challenged cassava (*Manihot esculenta* Crantz) suspension cells and leaves. Ann. Bot. 94, 87-97.

Housti, F., Andary, C., Gargadenne, A., Amssa, M., 2002. Effects of wounding and salicylic acid on hydroxycinnamocyl malic acids in *Thunbergia alata*. Plant. Physiol. Biochem. 40, 761-769.

Khripach, V., Zhabinskii, V. N., Groot, A. D., 1999. Brassinosteroids. A New Class of Plants Hormones. Academic Press. 456 p.

Kim, K. K., Seo, S. S., Kim, J. E., Sung, S. K., Kwan, J. S., An, G., Kim, W. T., 2001. Two polyphenol oxidases are differentially expressed during vegetative and reproductive development in the Fuji apple. Plant Sci. 161, 1145-1152.

Kim, T. W., Wang, Z. Y., 2010. Brassinosteroid signal transduction from receptor kinases to transcription factors. Annu. Rev. Plant Biol. 61, 681–704.

Krishna, P., 2003. Brassinosteroid-mediated stress responses. J. Plant Growth. Regul. 22, 289–297.

Kuwabara, T., Katoh, Y., 1999. Involvement of the binuclear copper site in the proteolytic activity of polyphenol oxidase. Plant Cell Physiol. 40, 1029-1035.

Marigo, G., 1973. On one fractioning methods and estimations of phenolic compounds in the vegetables. Analyse. 2, 106-110.

Michael, D. G., Spencer, G. F., Rohwedder, W. K., Mandava, N., Worley, J. F., David Warthen, J., Steffens, G. L., Flippen-Anderson, J. L., Carter Cook, J., 1979. Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen. Nature. 281(5728), 216-217.

Mohammed, N. A., Sarah, M. A., 2016. Effect of 28-hom obrassinolide on the performance of sensitive and resistant varieties of *Vigna radiata*. Saudi J. Biol. Sci. 23, 698–705.

Nakashita, H., Yasuda, M., Nitta, T., Asami, T., Fujioka, S., Arai, Y., Sekimata, K., Takatsuto, S., Yamaguchi, I., Yoshida, S., 2003. Brassinosteroid functions in broad range of disease resistance in tobacco and rice. Plant J. 33, 887-898.

Navodit, G., Prabir, K. P., 2014. Induction of systemic resistance in tomato by fruit extracts of *Azadirachta indica.* Rev. Literature. 2, 1-27.

Ngadze, E., Icishahayo, D., Coutinho, T. A., Van der Waals, J. E., 2012. Role of polyphenol oxidase, peroxidase, phenylalanine ammonia lyase, chlorogenic acid, and total soluble phenols in resistance of potatoes to soft rot. Plant Dis. 96, 186-192.

Ngoko, Z., 1994. Les maladies du maïs dans les hauts plateaux du Cameroun .IRA/MINREST Yaoundé. 22 p.

Okoruwa, A.C., 1995. Enhancing maize processing and utilization in West and Central Africa. Contribution to Food Self-sufficiency: maize research and development in West and Central Africa in Apraku (Eds) Cotonou. pp 10-20.

Ondobo, M. L., Effa, O. P., Manga, N. J., Djoko, K. J. C., Djocgoue, P. F., Omokolo, N. D., 2017. Estimation of necrotic length, phenol content, peroxydase and polyphenoloxydase activity in cacao plants (*Theobroma cacao* L.) after *Phytophthora megakarya* inoculation. Int. J. Agron. Agric. Res.10(3), 26-36.

Pirovani, P. C., Heliana, A. S. C., Regina, C. R., Dayane, S. G., Fatima, C. A., Fabienne, M., 2008. Protein extraction for proteome analysis from cacao leaves and meristems, organs infected by *Moniliophthora perniciosa* the causal agent for the witches broom diseases. Electrophor. J. 29, 2391-2401.

Siegfried, W., Viret, O., Huber, B., Wohlhauser, R., 2007. Dosage of plant protection products adapted to leaf area index in viticulture. Crop Prot. 26, 73-82.

Steven, D. C., 1998. Brassinosteroids: Essential regulators of plant growth and development. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49, 427–451.

Tchumakov, E. E., Zaharova, J. J., 1990. Influence statistique du développement des maladies. In Dommages causés par les maladies aux cultures agricoles. Ed. Agronome, Moscou. pp.5-60.

Vaiyapuri, B., Divya, V., Jean, C., Natarajan, S., 2012. Plant b-1,3-glucanases: their biological functions and transgenic expression against phytopathogenic fungi. Biotechnol. Lett. 34, 1983–1990.

Van Kammenn, A., Broumer, D., 1964. Increase of polyphenoloxidase activity by a local virus infection in uninoculated parts of leaves. Virology. 22, 9-14.

Wititsuwannakul, D., Chareonthiphakorn, N., Pace, M., Wititsuwannakul, R., 2002. Polyphenol oxidases from latex of *Hevea brasiliensis*: Purification and characterization. Phytochemistry. 61, 115-121.

Yu, J. Q., Huang, L. F., Hu, W. H., Zhou, Y. H., Mao, W. H., Ye, S. F., Nogues, S., 2004. A role for brassinosteroide in the regulation of photosynthesis in *Cucumis sativus.* J. Exp. Bot. 55(399), 1135-1143.

Zhu, J. Y., Sun, Y., Wang, Z. Y., 2012. Genome-wide identification of transcription factor-binding sites in plants using chromatin immunoprecipitation followed by microarray (ChIP-chip) or sequencing (ChIP-seq). Methods Mol. Biol. 876, 173-188.

Zhu, Q., Maher, E. A., Masoud, S., Dixon, R. A., Lamb, C. J., 1994. Enhanced protection against fungal attack by constitutive coexpression of chitinase and glucanase genes in transgenic tobacco. Bio-Technology. 12, 807-812.

Zulkarami, B., Qurban, A. P., Mohd, R. I., Halimi M. S., Mondal, M. A. M., Umme, A. N., Islam, Md. R., 2014. Rice yield improvement by foliar application of phytohormone. J. Food, Agric. Environ. 12 (2), 399-404.

How to cite this article:

Kuate Tueguem, W. N., Ngoh Dooh, J. P., Essono Obougou, G. G., Ndongo, B., Atindo, S. T., Heu, A., Tene Tayo, P. M., Ambang, Z., 2018. Effects of foliar application of 24-epibrassinolide on growth and induction of resistance of maize plants to helminthosporiosis on the field. Int. J. Curr. Res. Biosci. Plant Biol. 5(9), 61-73. **doi:** https://doi.org/10.20546/ijcrbp.2018.509.006