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Polyamines: Induction and Effect on Rust Disease Control of Bean

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ABSTRACT

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Two experiments were conducted to evaluate the potential effect of polyamine induction (putrescence, spermidine and spermine) in bean plants on control of rust disease caused by *Uromyces appendiculatus* under both greenhouse and field conditions. Three concentrations, 0.5, 1.0 and 1.5 mM of putrescine, ornithine and arginine were used as precursors of diamine putrescence, the triamine spermidine and tetraamine spermine. In the greenhouse, the disease severity and the number of pustules and uredospores were substantially reduced by all tested concentrations of polyamines precursors. At the same time, spraying of precursors of polyamines led to significant increases in levels of free putrescence, spermine and spermidine during the 21 days of experimental period. Meanwhile, treated leaves also exhibited significant increase in the amount of soluble conjugated putrescence, spermidine and spermine. These changes were accompanied by significant increase in the activity of soluble ornithine decarboxylase (ODC) and polyamine oxidase (PAO) during the 21 days of experimental period. Polyamines concentrations, ODC activity and PAO activity were greatly decreased in inoculated control treatment. Levels of free, soluble conjugated polyamines, ODC activity and PAO activity were greatly increased following treatment with putrescine at 1 mM. Moreover, significant increases in activities of plant defence related enzymes, peroxidase, polyphenoloxidase, free and conjugated phenol contents and pathogen related proteins were recorded in treated plants compared with untreated infected plants. In field trials, twice application of precursor of polyamines led to a significant reduction in rust disease infection and the number of pustules and uredospores during growth periods. Significant increases in growth characters and final yield were obtained in comparison with the untreated control. The most effective treatment was putrescine at 0.5 and 1.0 mM. However, high effect was also obtained by ornithine and arginine at 1.0 and 1.5 mM. It is concluded that the potential activity of these compounds may be related to the accumulation of diamine putrescence and polyamines spermidine and spermine and specifically to their toxicity and role in resistance. It was suggested that induction of polyamines may become a promising new approach for controlling rust disease of bean plants.

Key words: Bean, putrescence, ornithine, arginine, spermidine and spermine, rust and *Uromyces appendiculatus*

INTRODUCTION

Rust of bean (*Phaseolus vulgaris* L.) caused by *Uromyces appendiculatus* (Pers.,) Link is a serious disease in many parts of the world [18, 3, 14] . Bean rust mainly affects leaves and pods and causes yield losses closed to 60.0% depending on the severity and earliness of the infection [9]. Increasing concern about both the environmental consequences of repeated fungicide applications and rapid development of resistant fungal strains have limited the chemical control options of plant diseases. Therefore, recent efforts have been directed primarily toward identifying new control measures that could be effective, reliable and safe for the environment.

Polyamines (PAs) are essential for normal growth and development of plants and also in the regulation of several cellular and molecular functions [27]. Polyamines occur in plants in free form, bound electrostatically to negatively charged molecules, and conjugated to small molecules and proteins [22]. The major forms of polyamines are putrescine, spermidine and spermine. The three polyamines are normal constituents of eukaryotic and prokaryotic cells and are important regulators of growth and differentiation as well as in plant responses to stress. The starting point for polyamine biosynthesis in plants is the basic amino acids ornithine and arginine, which are decarboxylated by ornithine decarboxylase (ODC) and arginine decarboxylase (ADC), respectively to yield putrescine. Putrescine then serves as the substrate for the formation of spermidine and spermine syntheses [6, 29]. Since fungi possess only the ODC route to putrescine, specific control of plant fungal diseases through the inhibition of this enzymes has been recognized [12,30]. Indeed, various ODC inhibitors have been shown to reduce pathogenic fungal growth and infection including difluoromethylornithine (DFMO) for *Phytophtora infestans* [31] and *Sclerotinia sclerotiorum* [11 12]. Also, putrescine anologue 1- aminooxy-3-aminopropane caused inhibition of ODC in S. *sclerotiorum* [12]. Regarding plant diseases of fungal origin, inhibitors of polyamine biosynthesis also exerted powerful fungicide activity [24].

Polyamine levels are also known to change in plants in response to biotic stress, e.g. pathogen infection [29]. Von Ropenack and Schulze [28] found that polyamine conjugate, ρ - coumaroyl-hydroxyagmatine accumulated to a significant level in a resistant barley variety challenged with powdery mildew. The authors speculated that it could accumulate close to fungal infection structures. Polyamine conjugate was found to exhibit significant effect against the powdery mildew fungus either *in vitro* or *in vivo*. At the same time, antifungal activity was also exhibited by two spermidine conjugates isolated from roots of *Allium* species against *Fusarium* [8] and three from pollen of *Quercus alba* against powdery mildew pathogen [32]. However, little is known about the role of polyamine in plants defense and in the generation of disease resistant. Most of studies on polyamines (putrescine, spermidine and spermine) have been implicated in a variety of stress responses in plants. Typically, the cellular levels of either putrescine or spermidine or both are increased in response to the application of different forms of abiotic stress to cell cultures as well as whole plants. In light of the present work, it was decided to synthesize and induce diamine, putrescine and polyamines, spermidine and spermine by using precursor putrescine, ornithine and arginine and to examine their role in control of rust disease caused by *Uromyces appendiculateis* (Pers.,) in bean plants .

MATERIALS AND METHODS

Greenhouse experiments

Bean seedlings (30 days old) of cultivar ‘ Bronco ’ highly susceptible to rust disease were grown in 25 cm diameter plastic pots (5 plants / pot) filled with loamy and sandy soil (1:1, W/W) and watered daily to field capacity. Plants were kept in a greenhouse under 25^{0}C and 16 h photoperiod.

Uredospores of *U. appendiculatus* were obtained from naturally infected bean plants in the above mentioned greenhouse and collected soon after the onset of sporulation by scraping pustules. Plants were artificially inoculated by painting a suspension of *U. appendiculatus* uredospores (25 spores/100 ml) in 0.01% tween 80 on shoot surfaces [33]. Inoculated plants were enclosed with polyethylene bags for 48 h to provide the high humidity for enhancing uredospores germination.

Treatment with precursors of di- and polyamines

Polyamine precursors used were putrescine, ornithine and arginine (Sigma chemicals). Bean plants (30 days old) were sprayed with 0, 1.0 and 1.5 mM solution of each precursor of polyamine in distilled water and 0.01% tween 80, 48 h before inoculation with rust fungus. Five replicates and twenty plants were used for each treatment.

Disease assessment

Infection severity was assessed 7, 14 and 21 days after treatment using linear scales [25]. The percentage disease severity of all leaves on the marked plants was estimated by: 0 = No rust; 1 = 1% of shoot area covered by rust pustules; 2 = 5% of shoot area covered by rust

pustules; 3 = 10% of shoot area covered by rust pustules; 4 = 25% of shoot area covered by rust pustules; 5 = 50% or more of shoot area covered by rust pustules. In this respect, five random plants in each replicate were examined, and percentage of disease severity was calculated according to the following formula:

$$P = \text{Sum of } (n \times v) / 5N \times 100\%$$

P= Percentage of disease severity, n=Number of shoots in each category, V= Numerical value of each category and N= Total number of shoots in sample.

Number of pustules per leaf was counted and spores/cm^2 were calculated.

Ten mature leaves of five plants from each treatment were separately collected 21 days after treatment to determine the photosynthetic pigments, chlorophyll a and b as well as carotenoids according to the method described by Wettesein [34] at wave length of 662, 664 and 440 nm, respectively.

Chemical analysis

After 1,7,14 and 21 days of treatment with 1 mM concentration of putrescine, ornithine and arginine, five leaves/ plant were separately collected, frozen for 36 hrs, dried and powdered. Generally, 100 mg of dried samples was employed for analysis.

Polyamine analysis: Free and conjugated polyamines were extracted and hydrolysed using the method described by Slocum and Galston [26]. This yielded a non – hydrolysed perchloric acid (used at 10%) supernatant, containing the free polyamines, and the hydrolysed supernatant and pellet fractions, containing polyamines liberated from various types of conjugates. Polyamines were extracted with 2 ml of 0.5M $HClO_4$ overnight at room temperature, derivatized with benzoyl chloride and quantitated with high performance liquid chromatography (HPLC) using standard chemicals (Sigma chemicals). The derivatization procedure was basically according to the method of Flores and Galston [10]. Separation and quantification of derivatized polyamines were performed with a Shimadzn Lc-6A HPLC equipped with a UV detector. The analytical condition was as follows: 6×150 mm in column size; 45℃ column temperature; 64% methanol mobile phase and detection on 254 nm.

Enzyme assays: Ornithine decarboxylase (ODC), polyamine oxidase (PAO), peroxidase and polyphenoloxidase activity was determined after 1, 7, 14 and 21 days of treatment. ODC and PAO activity assays were performed as described previously [36]. Peroxidase activity was assayed as described by Allam and Hollis [1]. One unit of peroxidase was expressed for the change in absorbance at 470 nm for 2 min at 25℃ / g fresh weight. Polyphenoloxidase activity was quantitatively determined according to the method described by Matta and Dimond[23]. One unit of polyphenoloxidase was expressed as the change in absorbance at 420 nm for 30 min at 25℃/ g fresh weight.

Phenols: Free and conjugated phenols in leaves treated with 1 mM concentration of tested compounds were determined, after 15 days of plant spraying, according to the A.O.A.C.[2] using the Folin – Danis reagent. Phenols were identified by high performance liquid chromatography (HPLC) using a reverse phase C8 column and compared with as a standard (Sigma chemicals).

Protein content: Protein content in leaves treated with 1 mM concentration of putrescine, ornithine and arginine was determined after 15 days of spraying. Protein extraction was carried out according to Bollag & Edelstein[5], and separated by polyacrylamide gel electrophoresis (SDS-PAGE). Electrophoresis was performed according to Laemmli [16] using 10 % acrylamide in the separating gel and 3 % in the stacking gel. Protein samples (20-30 µg) in a total volume of 16 µg (8 µg of sample in extraction buffer + 8 µg 2X denaturing buffer) were denatured for 3 min in a boiling water bath, cooled, centrifuged and 15 µg was applied. Electrophoretic separation was carried out using mini gel unit at 60 volt for four hours. The gel was stained with silver stain according to Wray *et al*. [35] and distained with methanol solution. Molecular weights of polypeptide bands (KD) were calculated from a calibration curve of low molecular weight marker kit of *Phramacia* (Uppsala, Sweden).

Field experiments

The field experiments were conducted at Gezerit El-Dahab, Giza govarnorate, during 2003 and 2004 seasons under natural infection conditions. A randomized complete block design with five replicates for each treatment was used. Each replicate consisted of 50 plants. Each plot (20 m^2) consisted of 5 m long rows spaced 0.5 m apart. Fifteen bean seeds cv. ‘Bronco’ were sown per row. All polyamine precursors at the effective concentration of each compound were applied as foliar application at two periods, i.e. at 3-4 leaf growth stage and at 10-12 leaf growth stage. The control was treated with water. Disease severity was estimated and recorded during growth periods as previously mentioned. Number of pustules and spores /cm^2 were also recorded. At harvesting stage, growth rate and yield were determined.

Statistical analysis: Data were subjected to analyses of variance and treatment means were compared by an approximate Student’s t-test ($P<0.05$).

RESULTS

Greenhouse experiment

Evaluation of different polyamine precursors for control of rust disease infection of bean plants under artificially infested conditions clearly indicated that rust infection was reduced by 0.5, 1.0 and 1.5 mM of putrescine, ornithine and arginine, 48 h before inoculation (Table 1). Few differences were obtained between putrescine, ornithine and arginine applications and low (0.5 mM) and high (1.5 mM) concentrations. Greatest control was achieved using 1.0 and 1.5 mM of putrescine, which reduced rust disease infection of bean to 1.21 and 1.01 %, respectively, compared to 33.3 at the final assessment. At the same time, good results were obtained with ornithine and arginine treatments at 1.5 mM that reduced rust infection to 2.01 and 3.36 %, respectively, after 21 days of treatment. Data presented in Table 1 indicated that all polyamine precursors significantly reduced the mean number of pustules/ cm^2 compared with untreated plants. Putrescine at 1.0 and 1.5 mM, was effective in reducing the number of pustules/ cm^2 to 0.66 and 0.53 compared to 24.6 in the control in the final assessment. The same trend of response was also obtained with ornithine and arginine at 1.0 and 1.5 mM. As far as spraying polyamine precursors on bean plants 48 h before inoculation is concerned, all of them significantly decreased the spores number on leaf compared with infected control plants (Table 1). Significant reduction in spores number was obtained on bean leaves sprayed with putrescine. Ornithine and arginine at 1.5 mM also greatly reduced the uredospore number.

Data in Table 2 revealed that photosynthetic pigments: chlorophyll-a, chlorophyll-b and carotenoides were greatly decreased in bean plants inoculated with *U. appendiculatus* compared with untreated control. However, photosynthetic pigments were increased in plant leaves sprayed with 0.5, 1.0 and 1.5 mM of putrescine, ornithine and arginine, 48 h before inoculation compared with untreated control. Putrescine at 1.0 and 1.5 mM concentrations showed the highest effect on increasing photosynthetic pigments. In addition, similar response was also obtained with ornithine and arginine at 1.5 mM .

Free and conjugated polyamines

Concentrations of both free and conjugated polyamine forms i. e. putrescine, spermidine and spermine in bean leaves following treatment with 1mM of putrescine, ornithine and arginine throughout the experimental period (21 days) were showed in Fig. 1 and 2. Concentrations of free polyamines, putrescine, spermidine and spermine, decreased in plants inoculated with *U. appendiculatus*, reaching 63.2, 20.5 and 24.2 nmol/g compared to 143.5, 35.1 and 64.2 nmol/g , respectively , in uninoculated plants after 21 days (Fig. 1). In general, concentrations of free putrescine and spermine followed by spermidine were greatly increased in leaves of plants inoculated and treated with all polyamine precursors. Indeed, large increases in concentrations of free putrescine, spermine and spermidine were detected in leaves treated with 1.0 mM putrescine, reaching 697.0, 523.5 and 384.5 nmol/g, respectively, after 21 days. Similar trend was also observed with ornithine treatment,

Table 1. Effect of polyamine precursors applied to bean plants, 48 h before inoculation with *Uromyces appendiculatus* on the rust disease severity (%), pustules and spores number

Polyamine precursor	Conc. (mM)	Rust infection[1] (%)			Pustules / cm^2			Uredospores / cm^2		
		7[2]	14	21	7	14	21	7	14	21
Control	---	8.65±0.78*	17.5±1.54	33.3±3.23	9.23	11.5	24.6	33.3±3.12	88.3±4.54	162.1±21
Putrescine	0.5	0.0	0.78±0.03	2.33±0.21	0.0	0.52	0.87	3.36±0.30	10.32±0.10	12.2±0.14
	1.0	0.0	0.38±0.87	1.21±0.40	0.0	0.21	0.66	0.06±0.14	0.11±0.01	8.12±0.1
	1.5	0.0	0.0	1.01±0.65	00	0.0	0.53	0.08±0.10	0.5±0.01	4.09±0.04
Ornithine	0.5	1.8±0.54	2.32±0.21	4.25±0.51	0.74	1.21	4.23	8.65±0.74	18.2±0.65	23.5±0.41
	1.0	0.0	1.45±0.23	3.21±0.20	0.0	0.87	2.45	5.35±0.87	9.65±0.35	14.6±0.25
	1.5	0.0	0.02±0.01	2.01±0.21	0.0	0.20	1.06	4.65±0.45	7.54±0.64	11.5±0.34
Arginine	0.5	2.32±0.89	3.21±0.21	6.23±2.54	1.33	1.35	7.65	16.2±1.02	21.3±1.32	33.3±1.21
	1.0	1.35±0.65	2.41±0.65	4.81±3.12	0.98	1.15	5.12	14.2±1.32	17.3±1.45	21.3±1.34
	1.5	0.98±0.1	2.12±0.52	3.36±2.14	0.54	0.98	4.23	10.5±0.75	12.8±0.78	18.6±1.0
LSD(P<0.05)		0.49	0.61	0.96	0.41	0.87	1.23	2.95	4.35	8.84

[1] Rust disease severity on bean was assessed using the following key: 0=No rust; 1 = 1% of shoot area covered by rust pustules; 2 = 5% of shoot area covered by rust pustules; 3 = 10% of shoot area covered by rust pustules; 4 = 25% of shoot area covered by rust pustules; 5 = 50% or more of shoot area covered by rust pustules.* Variable mean ± standard error of the mean; means of five replicates.

[2] Days after treatment.

Table 2. Effect of polyamine precursors applied to bean plants, 48 h before inoculation with *Uromyces appendiculatus* on chlorophyll-a, chlorophyll-b and carotenoids contents

Polyamines precursor	Conc. (mM)	Photosynthetic pigments (mg/ cm^2 fresh weight)		
		Chlorophyll-a	Chlorophyll-b	Carotenoids
Control (without inoculation)	---	4.27[1]	2.18	2.55
Control (inoculation)	---	1.05	0.01	0.34
Putrescine	0.5	4.87	2.65	2.93
	1.0	5.65	2.79	3.68
	1.5	7.12	3.08	3.97
Ornithine	0.5	4.75	2.56	2.97
	1.0	5.42	2.70	3.45
	1.5	6.24	2.89	3.65
Arginine	0.5	4.67	2.46	2.93
	1.0	4.87	2.66	3.26
	1.5	5.74	2.78	3.33
LSD(P<0.05)			0.53	0.23

[1] Variable mean ± standard error of the mean

reaching the values of 542.3, 398.2 and 314.5 nmol/g, respectively. Increase in putrescine, spermine and spermidine after 21 days of plant treatment with arginine at 1 mM was also observed. Similar results were obtained with conjugated polyamines. Concentrations of conjugated putrescine, spermidine and spermine were greatly decreased during the experimental period in plants inoculated with *U. appendiculatus*, reaching 51.2, 22.2 and 0 nmol/g compared to 93.2, 71.2 and 27.2 nmol/g, respectively, in uninoculated treatment after 21 days (Fig. 2). In general, the conjugated polyamines of putrescine and spermidine were greatly increased in leaves treated with polyamine precursors during growth periods. Meanwhile, slight increase in spermine was observed. Significant increase in putrescine and spermidine was found in leaves treated with putrescine , reaching 675.2 and 572.3 nmol/g, respectively, after 21 days. Putrescine and spermidine concentrations were also increased after 21 days following treatment with ornithine or arginine at 1 mM.

Activities of polyamine biosynthetic enzymes

Activity of soluble ornithine decarboxylase (ODC) and polyamine oxidase (PAO) in leaves treated with 1 mM of different polyamine precursors and inoculated with *U. appendiculatus* during 21 days of experimental period was shown in Fig. 3. In leaves inoculated with *U. appendiculatus*, soluble ODC activity was slightly increased after 7 days of treatment [83.2 Pmol (mg protein)/ h], but decreased thereafter. The untreated control remained more or less the same during the experiment. However, activities of both enzymes greatly increased in leaves treated with putrescine, ornithine and arginine during growth periods. Significant increase in soluble ODC activity was found in leaves treated with putrescine, reaching 142.2 Pmol (mg protein)/ h after 21 days of treatment. Activity of ODC also increased [133.3 Pmol (mg protein)/ h] after 21 days following treatment with ornithine. The ODC activity in leaves following treatment with arginine at 1 mM also was increased [119.8 Pmol (mg protein)/ h].

The pattern of PAO activity was similar to that of ODC activity. In leaves inoculated with *U. appendiculatus*, the soluble PAO activity was slightly increased after 7 days [36.7 Pmol (mg protein)/ h]. It greatly declined thereafter. The activity of PAO remained more or less constant in control during the experiment. Meanwhile, both enzyme activities greatly increased in leaves treated with putrescine, ornithine and arginine during growth periods. Large and significant increase in soluble PAO activity was found in leaves treated with putrescine, reaching 178.0 Pmol (mg protein)/ h after 21 days. Activity of PAO also increased [154.6 Pmol (mg protein)/ h] after 21 days of treatment with ornithine. Soluble PAO activity in leaves following treatment with arginine at 1 mM was similarly increased [150.2 Pmol (mg protein)/ h].

Peroxidase and polyphenoloxidase activity.

Activity of defense-related enzymes peroxidase and polyphenoloxidase in leaves treated with 1 mM concentration of different polyamine precursors and inoculated with *U. appendiculatus* during 21 days after treatment was illustrated in Fig. 4. Peroxidase activity was greatly decreasd in plants inoculated with *U. appendiculatus*, reaching 2.01 unit in comparison with untreated control (6.87 unit) after 21 days of treatment. On the contrary, activity of peroxidase was significantly increased in leaves following treatment with putrescine, ornithine and arginine reaching, 30.5, 28.3 and 28.5 unit,

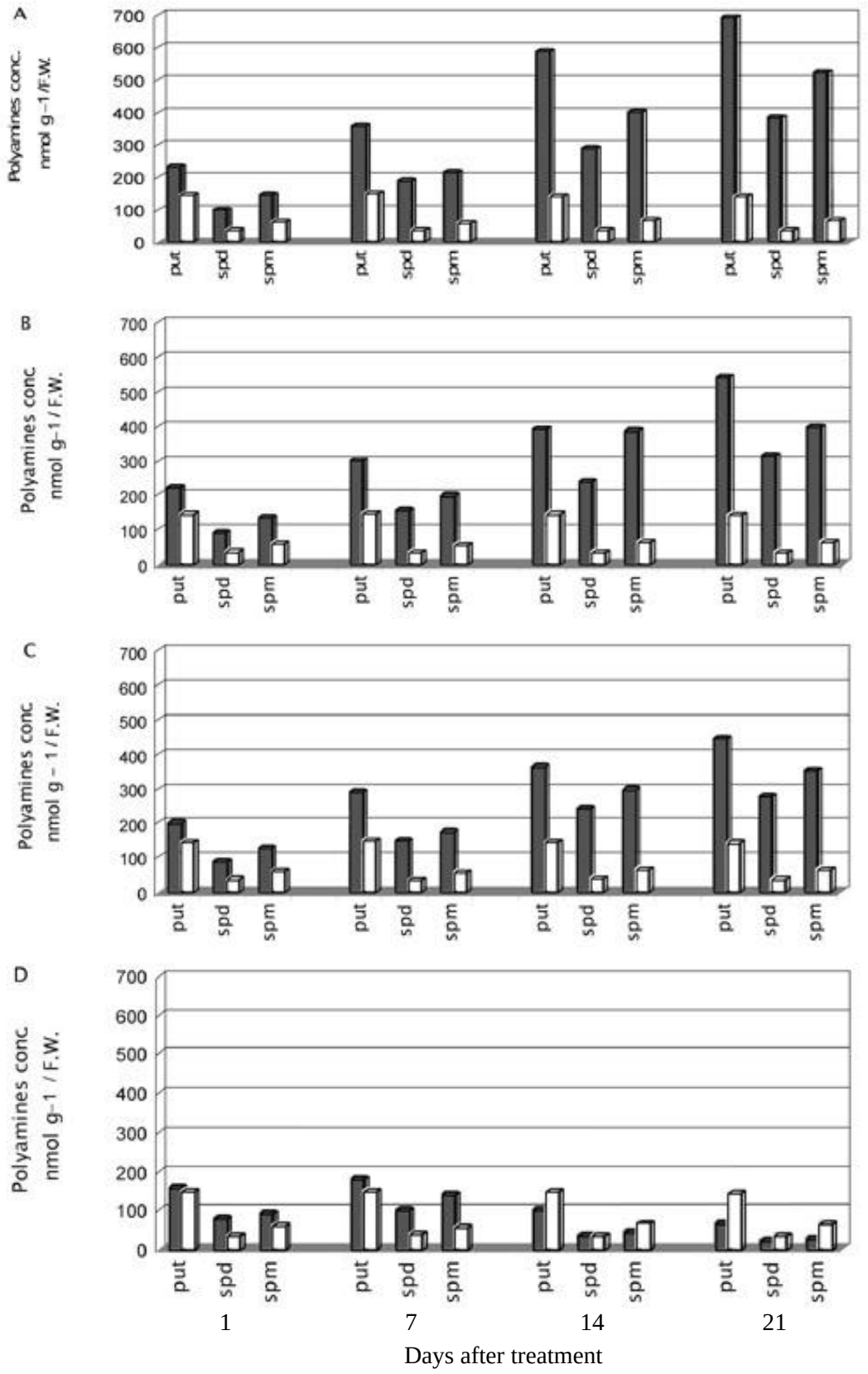

Fig. 1. Free polyamines concentrations, putrescine (put), spermidine (spd) and spermine (spm) in bean leaves following treatment with 1 mM of putrescine (A), ornithine (B) and arginine (C) in comparison with inoculated control (D) . Inoculated plants ( ) Untreated control (□).

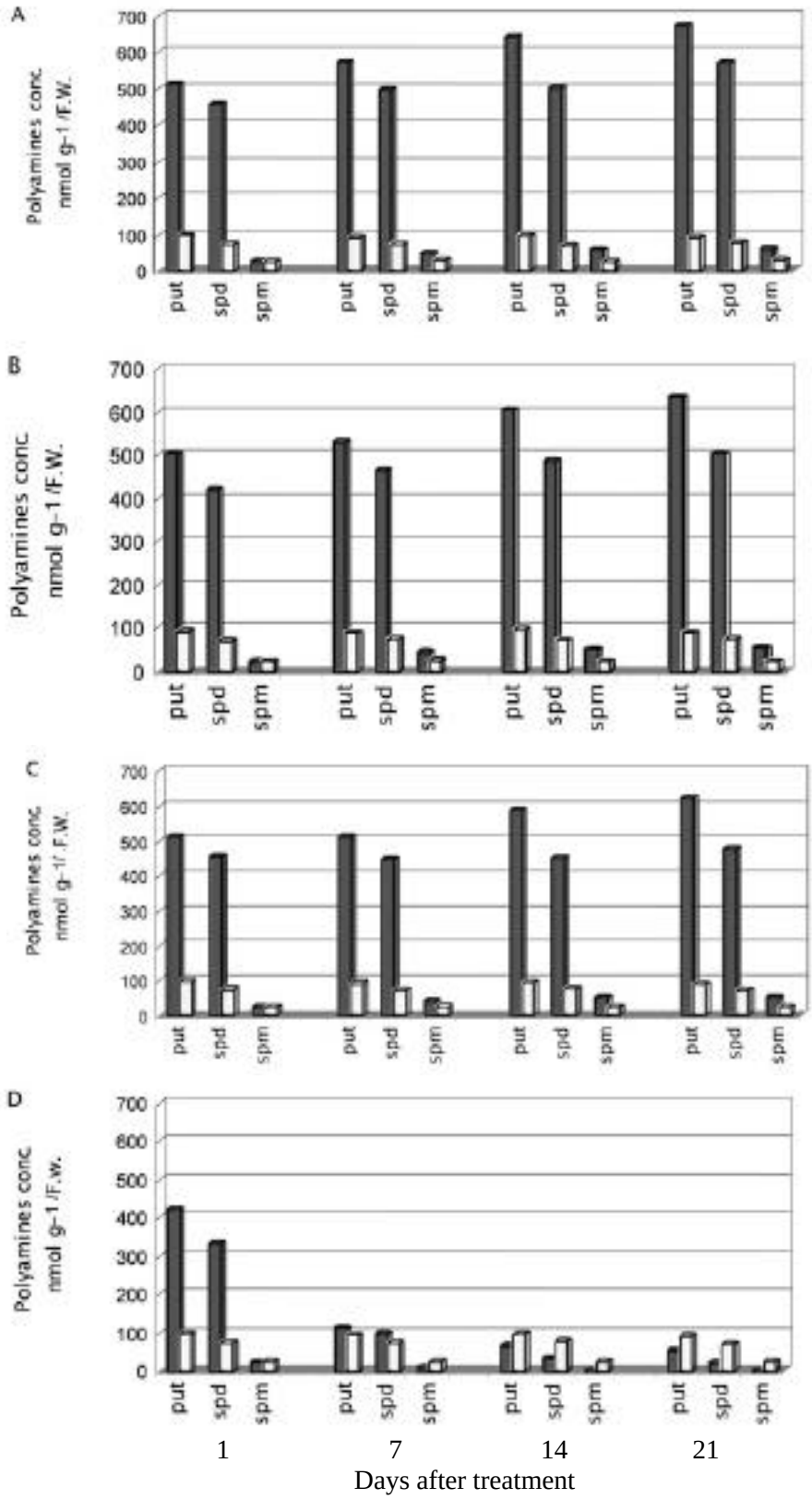

Fig. 2. Conjugated polyamines concentrations, putrescine (put) , spermidine (spd) and spermine (spm) in bean leaves following treatment with 1 mM of putrescine (A), ornithine (B) and arginine (C) in comparison with inoculated control (D). Inoculated plants( ) Untreated control (□).

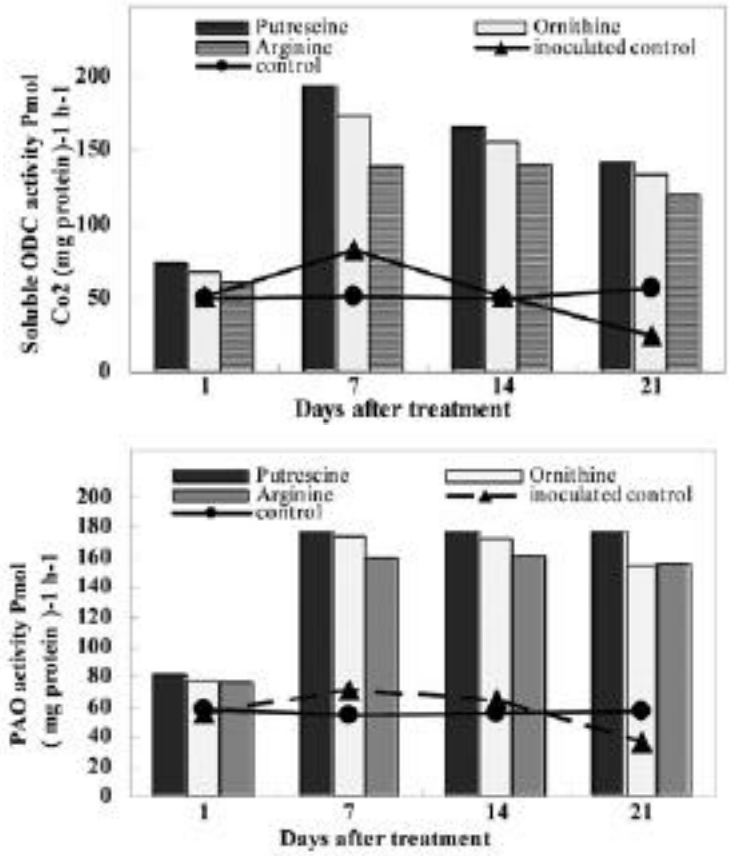

Fig. 3. Activity of soluble ornithine decarbodylase (ODC) and polyamine oxidase (PAO) in bean leaves following treatment with 1 mM of putrescine, ornithine and arginine in comparison with inoculated control and untreated control.

respectively, after 21 days of treatment .

Data also show that the pattern of polyphenoloxidase activity was similar to that of peroxidase activity. Polyphenoloxidase activity was greatly decreased in plants inoculated with *U. appendiculatus* during plant growth, reaching 0.054 unit in comparison with untreated control (0.116 unit) after 21 days of treatment. On the contrary, polyphenoloxidase activity in leaves treated with putrescine, ornithine and arginine was significantly increased. Activity of polyphenoloxidase was greatly increasd in treated leaves with putrescine and ornithine, reaching 0.642 and 0.601 unit, respectively, after 21 days of treatment. Moreover, polyphenoloxidase activity was significantly increased in leaves following treatment with arginine at 1 mM, reaching 0.587 unit after 21 days of treatment.

Free and conjugated phenols contents

There were prominent differences between untreated control, inoculated and polyamine precursor treatments (Fig. 5). Free and conjugated phenol contents greatly decreased in plants inoculated with *U. appendiculatus* when compared with control plants. Polyamine precursors sprayed on bean plants, resulted in an increase in free phenols content. For putrescine, the greatest increase in free phenol contents (22.3 mgcatechol/g) was obtained compared with untreated (15.67 mgcatechol/g) and inoculated (9.5 mgcatechol/g) plants. Ornithine and arginine also caused the increase of free phenol contents to 18.5 and 17.3 mg/catechol/g, respectively. Similar trend was observed in conjugated phenol content, which increased in all plants treated with polyamine precursors in comparisn with untreated control. Among the tested polyamine precursors , putrescine caused the greatest increase in conjugated phenols content (12.3 mgcatechol/g) in comparison with untreated (7.5 mgcatechol/g) and inoculated plants (3.12 mgcatechol/g). It was noticed that, application of ornithine and arginine led to significant increase in conjugated phenol contents (10.2 and 9.2 mgcatechol/g, respectively).

Pathogen related protein

The electrophoretic patterns (SDS – PAGE) of the crude proteins of treated leaves were compared with those

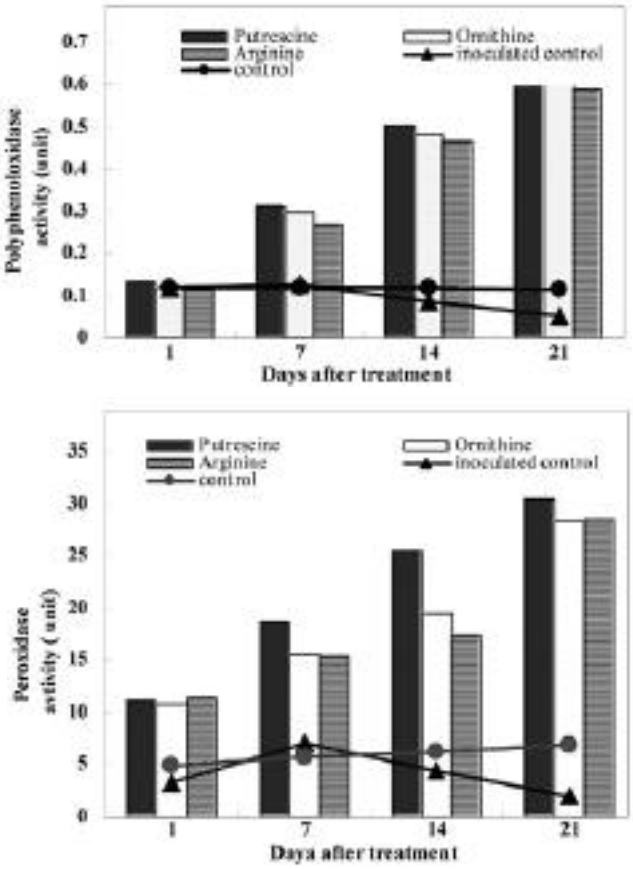

Fig. 4. Activity of peroxidase and polyphenoloxidase in bean leaves following treatment with 1 mM of putrescine, ornithine and arginine in comparison with inoculated control and untreated control.

of untreated control (Fig. 6 and Table 3). Seven protein banding patterns were detected in untreated control which was characterized by 37.4 KD total amount and 3.314 maximum optical density. Meanwhile, five protein banding patterns were observed in infected leaves with 20.8 KD in total amount and 1.951 in maximum optical density. However, the banding patterns of protein revealed high levels of polymorphism (or specific markers) in leaves treated with polyamine precursors. The number of protein banding patterns scored for plants treated with any of polyamine precursors was about 10 bands. For putrescine, the greatest increase in total amount (49.05 KD) and in optical density (3.320) was obtained. However, 44.78 and 43.68 KD of total amount and 4.693 and 4.383 of optical density were obtained by ornithine and arginine, respectively.

Field experiments

Significant differences ($P < 0.05$) were obtained among treatments of polyamine precursors and untreated control concerning control of rust disease on bean plants (Table 4). Analysis of data indicated that all polyamine precursor treatments significantly reduced rust disease severity in 2003 and 2004 seasons. Greatest control was achieved using 0.5 and 1.0 mM of putrescine, which reduced rust disease infection of bean by 2.62 and 2.48% and 1.02 and 1.95% compared to 19.3 and 18.06 % in untreated control after 90 days of planting in 2003 and 2004, respectively. Data also showed that when ornithine and arginine were applied at 1.5 mM, 1.51 and 2.32 % and 2.78 and 2.68 % disease incidence, respectively ,were recorded on bean plants after 90 days of planting in both seasons.

Plants treated with polyamine precursors reduced pustule and spore number of rust on bean plants in comparison with untreated plants (Table 5). Pustule as well as spore number were consistently lower when plants were treated with putrescine at 0.5 or 1.0 mM. Plants treated with ornithine at 1.5 mM showed reduced number of the pustules and spores. Arginine at 1.5 mM signification reduced both pustule and spore number on leaves. Data presented in Table 6 indicated that the application of different polyamine precursors affected growth (plant height and weight) and yield of bean plants in comparison with untreated plants in both seasons. The highest values of plant height and dry weight were from bean sprayed with putrescine at 1.0 mM. Ornithine and arginine at 1.5 mM also increased plant height and dry weight. Check plants had the lowest growth parameters in both seasons. As for pods yield/ plant, the highest increase was obtained by application of putrescine at 1.0 mM which increased it to 15.5 and 16.6 g/plant compared to 3.21 and 3.33g/ plant, respectively, in control in both seasons. Moreover, data revealed that spraying plants with ornithine and arginine at 1.5 mM, had a positive effect on increasing pod yield / plant (13.2 and 14.3 g/plant) and (11.45 and 11.5 g/ plant) , respectively, in both seasons.

DISCUSSION

The present experiments showed that all the polyamine precursors examined are intimately involved in rust disease control of bean plants under both artificially and naturally infested conditions. This effect was achieved through decreasing the disease severity and number of pustules and uredospores/cm^2 of bean leaves under artificially infested conditions. Results showed that photosynthetic pigments, chlorophyll-a, chlorophyll-b and carotenoides were increased compared with untreated plants. On the other hand, these pigments were greatly decreased in inoculated plants. Greatly elevated levels of free and conjugated polyamines in plants treated with

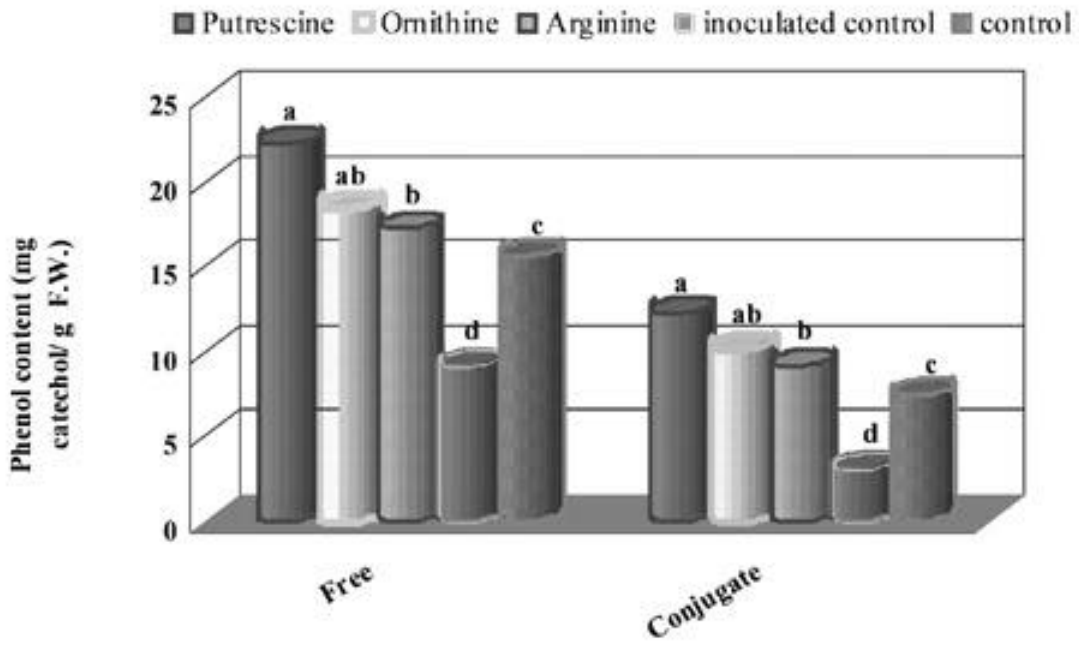

Fig. 5. Free and conjugated phenols contents in bean leaves following treatment with 1 mM of putrescine, ornithine and arginine in comparison with inoculated control and untreated control

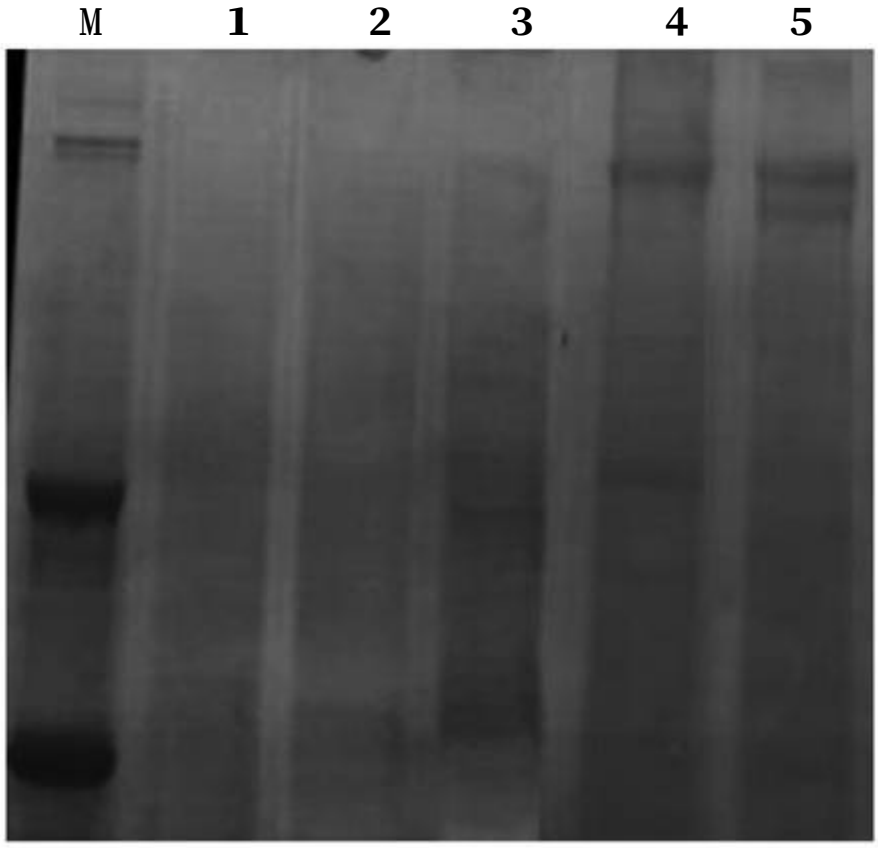

Fig. 6. SDS-PAGE patterns of protein profiles of bean leaves following treatment with 1 mM putrescine, ornithine and arginine in comparison with inoculated control and untreated control. Uuntreated control (1) Inoculated control (2), putrescine (3) , ornithine (4) and arginine (5). Low molecular weight standard protein marker is existed at (lane M).

Table 3. Molecular weight, amount and maximum optical density of SDS-PAGE patterns of protein of the bean leaves following treatment with 1 mM putrescine , ornithine and arginine

Lanes		Markers		Untreated control		Inoculated control		Putrescine		Ornithine		Arginine	
Rf	M.W.	Amount KD	Max OD	Amount KD	Max OD	Amount KD	Max OD	Amount KD	Max OD	Amount KD	Max OD	Amount KD	Max OD
R1	97	2.77	0.284					3.15	0.471	7.12	0.655	7.12	0.742
R2	71					3.58	0.465	5.6	0.609	3.45	0.354	6.52	0.675
R3	66	3.65	0.519	3.67	0.423								
R4	60							3.9	0.562	3.21	0.425	2.35	0.301
R5	51			7.51	0.603	5.06	0.545	5.92	0.645	3.54	0.435	3.45	0.312
R6	45	9.1	0.483					5.1	0.585	7.12	0.632	4.36	0.354
R7	41			5.53	0.541								
R8	38							8.21	0.855	6.45	0.679	3.21	0.324
R9	35			6.82	0.579	5.16	0.552						
R10	30	3.82	0.609					5.85	0.674	3.45	0.412	3.65	0.354
R11	25			4.69	0.567			3.9	0.637	3.56	0.435	3.56	0.341
R12	20.1			4.66	0.601	3.19	0.389	3.86	0.635	3.32	0.365	3.61	0.578
R13	14.4			4.56	0.582	3.81	0.305	3.56	0.321	3.56	0.301	3.41	0.402
Sum				37.4	3.314	20.4	1.951	49.05	5.320	44.78	4.693	43.68	4.383

polyamine precursors were observed. This may be attributed to a striking increase in activity associated with plant defense against rust pathogen in plants treated with putrescine, ornithine and arginine. Polyamine precursors treatment led to increase in both free and soluble conjugated diamine putrescine, the triamine spermidine and tetraamine spermine during the experimental span (21 days). Results also showed that free spermidine and spermine showed a gradual increase as compared to the control. Furthermore, greater increases in soluble conjugated putrescine and spermidin and slight increase in soluble conjugated spermine were observed. In contrast, levels of both free and conjugated polyamines were greatly decreased in infected nontreated control. The infection of tobacco leaves with *Erysiphe cichoracearum* and *Alternaria tenuis* was also reported to result in decreasing concentrations of free putrescine and spermidine [7]. Decrease in free spermine contents in sugarcane plants infected with *Ustiligo scitamine* has also been reported [17]. Leaves treated with various polyamine precursors led to increase in the activity of the polyamine biosynthetic enzymes ODC and PAO. The elevated levels of free and conjugated di-and polyamine in bean leaves were accompanied by increasing the polyamine biosynthesis, during the 21 days of experimental period. The increased enzyme activities can account for the increase in free putrescence, spermidine and spermine. Interestingly, free and conjugates di-and polyamines have been implicated in plant resistance to pathogen [29]. In the present study, three polyamines were also shown to reduce rust infection of bean plants. Recently, commercial spermidine analogues were reported to possess fungicidal activity and ability to control powdery mildew infection on barley and apple [20], and inhibitory effect on the growth of *Pyrenophora avenae* [19].

Spraying bean plants with polyamine precursors appeared to increase the activity associated with plant defense against pathogen as increased activity of peroxidase and polyphenoloxidase were observed. Increasing of peroxidase and polyphenoloxidase activity was associated with increasing resistance against infection by rust causal organism[15]. Free and conjugated phenols as well as pathogen related proteins (PR) have to increase following treatment. In fact, synthesis of phenols is important in many forms of resistance[12]. Hiraga *et al*.[13] proposed an attractive hypothesis that spermine might have a key role in the resistance of tobacco plants against virus infection by activating phytopathogenesis-related protein (PR) gene. In general, polyamine metabolism has long been known to be altered in plants responding to profound changes in plants interacting with fungal and viral pathogens [29, 6 & 30]. Polyamines conjugated to phenolic compounds, hydroxycinnamic acid amides (HCAAs), have been shown to accumulate in incompatible interactions between plants and a variety of pathogens, while changes in the diamine catabolic enzyme diamine oxidase suggest a role for this enzyme in the production of hydrogen peroxidase during plant defense responses [30]. Therefore, such leaves also exhibit greatly reduced infection by rust pathogen compared to control.

Under field conditions, two foliar spray of bean plants with putrescine, ornithine and arginine, reduced rust disease severity to minimum level and number of pustules and uredospores /cm^2 on bean leaves. Such results are in accordance with previous work on the spermidine analogue norspermidine[21] which was shown to provide

Table 4. Effect of foliar application of different polyamine precursors on the rust disease severity of bean plants grown in field under natural conditions

Polyamine precursor	Severity of rust disease (%) after sowing days					
	Season 2003			Season 2004		
	30	60	90	30	60	90
Untreated control	1.3±0.2[1]	4.5±0.3	19.3±0.3	1.3±0.2	4.5±0.3	18.3±0.6
0.5mM Putrescine	0.0	0.91±0.2	2.62±0.1	0.0	0.91±0.2	2.48±0.5
1.0 mM Putrescine	0.0	0.0	1.02±0.4	0.0	0.0	1.95±0.5
1.0mM Ornithine	0.0	1.26±0.4	3.36±0.3	0.0	1.30±0.4	3.11±0.3
1.5mM Ornithine	0.0	0.0	1.51±0.2	0.0	0.0	2.32±0.2
1.0 mM Arginine	0.5±0.01	1.32±0.4	4.65±0.6	0.5±0.01	1.56±0.4	3.21±0.4
1.5 mM Arginine	0.0	0.0	2.78±0.5	0.0	0.0	2.68±0.2
LSD (P<0.05)	0.15	0.30	0.65	0.13	0.36	0.58

[1] Rust disease severity on bean was assessed using the following key: 0=No rust; 1 = 1% of shoot area covered by rust pustules; 2 = 5% of shoot area covered by rust pustules; 3 = 10% of shoot area covered by rust pustules; 4 = 25% of shoot area covered by rust pustules; 5 = 50% or more of shoot area covered by rust pustules. *Variable mean ± standard error of the mean; means of five replicates.

Table 5. Effect of foliar application of different polyamine precursors on the pustules and *Uromyces appendiculatus* spores number on bean plants grown in field under natural conditions

Polyamine precursor	Pustules/ cm [2]			Uredospores / cm [2]		
	30[2]	60	90	30	60	90
Untreated Control	4.21	8.14	15.2	21.5	38.5	97.2
0.5mM Putrescine	0.0	1.5	3.21	0.0	3.54	12.5
1.0 mM Putrescine	0.0	0.0	1.65	0.0	0.0	5.14
1.0mM Ornithine	0.0	3.56	4.65	0.0	11.5	24.5
1.5mM Ornithine	0.0	0.0	2.65	0.0	0.0	19.8
2.0 mM Arginine	0.65	1.87	5.24	4.32	24.2	37.5
1.5 mM Arginine	0.0	0.0	3.21	0.0	0.0	23.5
LSD (P<0.05)	0.45	0.5 8	0.8 2	2.78	3.54	6.45

[1] Variable mean ± standard error of the mean; means of five replicates.
[2] Days after sowing

Table 6. Effect of foliar application of different polyamine precursors on the growth and yield of bean under natural conditions

Polyamine precursor	Season 2003			Season 2004		
	Plant height	Plant weight	Pods yield / plant	Plant height	Plant weight	Pods yield / plant
	(cm)	(g)	(g)	(cm)	(g)	(g)
Control	33.5[1]	5.78	3.21	35.8	5.84	3.33
0.5mM Putrescine	68.5	18.2	11.31	69.2	19.5	12.5
1.0 mM Putrescine	78.5	21.2	15.54	77.6	23.3	16.6
1.0mM Ornithine	59.5	16.5	10.84	60.2	17.3	11.5
1.5mM Ornithine	64.2	19.3	13.20	66.6	18.5	14.3
1.0mM Arginine	57.6	14.6	10.43	58.6	14.3	10.3
1.5mM Arginine	60.4	17.3	11.54	58.6	16.8	11.5
LSD (P<0.05)	2.34	1.32	1.02	2.52	1.38	1.13

[1] Variable mean ± standard error of the mean; means of five replicates.

early control of powdery mildew on spring barley and result in a great increase in plant growth and final yield. These increaments could be due to antisenescence effect of polyamines, and increase in stomatal number and organic solutes (soluble sugars)[4].

In conclusion, the present work showed that polyamine biosynthesis and induction in bean plants is related to rust disease control because polyamine possess antifungal activity,and increases peroxidase, polyphenoloxidase, phenolic compounds, PR proteins and resistance to rust in bean plants.

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摘 要

Haggag, W. M. 2005. 聚醯胺的誘導與防治菜豆 病的效果. 植病會刊 14:89-102. (Department of Plant Pathology, National Research Center, Dokki, Cairo, Egypt. E-mail: mkelany@link.net)

於溫室與田間試驗評估誘導菜豆植體中聚醯胺 (腐胺、亞精胺及精胺) 對防治由 *Uromyces appendiculatus* 引起菜豆 病的可能效應。以 0.5、1.0、1.5 mM 等三種濃度的腐胺酸、鳥胺酸及精胺酸做為二元胺的腐胺、三元胺的亞精胺及四元胺的精胺等的前驅物。這些聚醯胺前驅物的三種濃度在溫室中大致上均能降低菜豆 病的罹病度與 斑及冬孢子的數目。在 21 天的試驗中，噴佈聚醯胺前驅物可明顯增加植體內腐胺、亞精胺及精胺的量；同時，處理的葉片同樣表現相同的結果。這種改變仍是隨著植體內可溶性鳥胺酸去羧瓷 (ODC) 和聚醯胺氧化瓷 (PAO) 活性明顯的增加而增加。聚醯胺的濃度、ODC 和 PAO 的活性在接種對照組植體內明顯降低，然而可溶性聚醯胺、ODC 和 PAO 的活性在處理 1.0 mM 的腐胺酸後明顯增加。另外，處理植體中植物防禦相關酵素、過氧化酵素、多酚氧化酵素的活性、酚化物含量及病原相關蛋白亦明顯增加。田間試驗噴施二次聚醯胺前驅物亦明顯降低菜豆生長期間 病菌的入侵及 斑與冬孢子數量，並明顯增加生育狀態與最後產量。最有效的處理為0.5 和 1.0 mM 腐胺酸，然而 1.0 和 1.5 mM 的鳥胺酸及精胺酸亦較對照組有抑病效果。聚醯胺前驅物的防病效果可能與二元胺的腐胺和聚醯胺的亞精胺和精胺累積，特定毒性及抗病性等有關。因此，聚醯胺的誘導應可做為防治菜豆 病的新方法。

關鍵詞：菜豆、腐胺酸、鳥胺酸、精胺酸、亞精胺、精胺、 病、*Uromyces appendiculatus*