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In *vitro* Effect of Exogenous Cytokinin and Fungal Toxin on Germination Seeds of *Cicer arietinum* L.

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Abstract

In Algeria, chickpea (*Cicer arietinum* L.) is the second food legume produced after beans. Its cultivation has undergone a certain gradual evolution in terms of areas and consumption, and a regressive evolution in terms of productivity. Despite this importance, Chickpea is faced with many phytosanitary problems, the most damaging of which are caused by fungi. One of the factors that restricts the growth of Chickpea is fungal diseases which are the main biotic stresses. In the present study, the effect of different concentrations of Cytokinin and Fungal metabolic from Aspergillus fumigatus on the germination of two types of Chickpea (FLIP 85-55 and ILC 32-79) was investigated. At first, various pretreatments were done on the seeds, and after that, the seeds were sown in Petri dishes. The findings demonstrated that when the concentration of toxin (fungal metabolic) increased, the germination rate decreased. The type ILC 32-79 was less tolerant than the type FLIP 85-55. Contrarily, a prominent improvement in the rate of germination was caused by the cytokinin.

Keywords: *Cicer arietinum* L., germination, exogenous application of hormone, in *vitro*, fungal metabolic, Aspergillus fumigatus.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the widely consumed vegetables all around the world (Hossain, et al, 2010; Shaheena, et al, 2012). The nutritional value of Chickpea lies in the high amount of protein existing in its seed composition (Table 1), therefore, it is widely consumed as a substitute for animal protein (Shrestha, et al., 2011).

In Algeria, after the soybean, chickpea is the second popular food legume. A progressive change in its productivity has happened between 1980-1990, its total production between 1990 and 1994 came to be 208.785, 6 quintals with an average yield that remained relatively low which was 4.6 kg / ha (Anonymous, 2010). The production had significant instability and decrement, and the chickpea crop was affected by biotic and abiotic constraints (Shaban, 2013; Yosef Tabar, 2013).

It has been reported that favorable atmospheric conditions for the disease development might cause 50 to70% crop losses (Malik, et al. 1984). Sometimes, they may cause failure of the whole chickpea crop. Disease epidemics have been reported in different parts of the world (Nene, 1982; Aslam, 1984; Kaiser, 1992). Through applying the foliar and the seed dressing fungicides (Reddy, et al. 1984; Rauf, et al. 1996), using disease free seed, destructing plant disease debris (Pandey, 1986), and increasing the host plant resistance (Iqbal, et al .2002; Ahmad, et al, 2006), the fungal diseases can be effectually managed.

Fungal diseases are the most important biotic factors limiting chickpea production. These diseases often cause low production, and agro-order techniques related to seeding conditions (time and method of sowing, and seed quality). They also lead to low yields and poor seed quality, and in some cases may cause a loss of performance up to 100%, especially in susceptible varieties.

Cytokinin application has been known to regulate several plant growth aspects and developmental processes, including cell division, and apical dominance (Mok, et al. 2001; Davies, 2010). Cytokinin can also break stress-induced dormancy during the germination (Bozcuk, 1981).

However, so far there have been few detailed studies of growth regulators and toxins on their germination and interaction. The aim of the study was to evaluate the influence of cytokinin and toxin on two Algerian Chickpea cultivars.

Table 1. Main Biochemical Constituents

Composition	Percentage (%)
Carbohydrates	38-59
Proteins	24.0
Water	7.3
Lipids	5.2

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Materials and Methods

Plant Material

The seeds of chickpea variety used in this study were provided by the Technologic Institution of Large Cultures (ITGC), El Khroub, Constantine, Algeria. The experimental materials were comprised of two chickpea genotypes; ILC 3279 and FLIP 85-55(Fig. 1, Table 2).

Fig. 1. Photos of the studied chickpea varieties.

Table 2. Origins and Characteristics of the Studied Chickpea Varieties.

Variety	FLIP 85-55	ILC 32-79
Origin	Icarda-Syrie	Stepnoj Ex URSS
Characteristics Cultivation vegetative cycle Flowering maturity	Late	Late 150 days 210 days
Morphological characteristics grain color grain shape productivity yield Weight of 100 seeds (gr)	Yellow Round high in the absence of disease 38	Beige Round high in the absence of disease 26

Fungal metabolic sample

The fungal metabolic (Toxin) of *Aspergillus fumigatus* was obtained from the Applied Microbiology Laboratory, Faculty of Science, University of Constantine (Algeria), which had previously been isolated and developed on cultured DCS, and the entanglement was identified by electron microscopy (Fig 2) (Dehimat, et al. 2010).

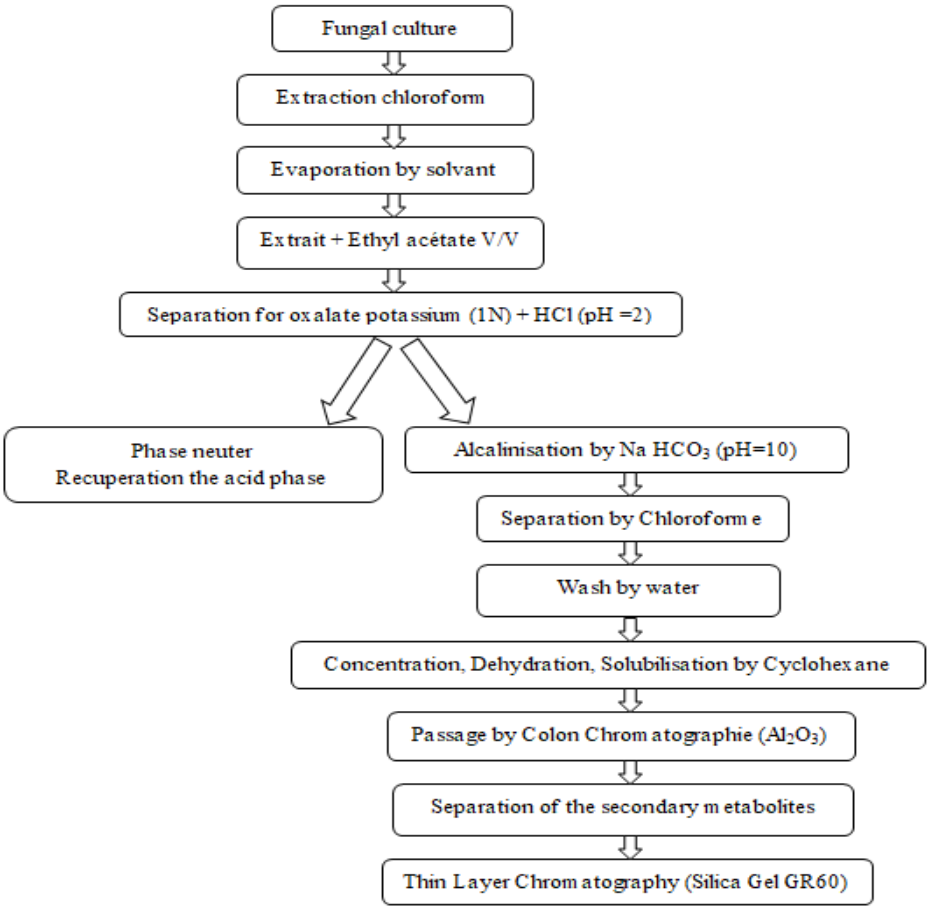

Fig. 2. Extraction and purification of secondary metabolic by products of *A. fumigates.*

Chemicals

Cytokinin was purchased from Sigma-Aldrich Chimie. Other chemicals were from Merck.

Soaking and germination of seeds

Seed varieties were soaked for one hour at different concentrations of fungal metabolic, and then washed with sterile distilled water to remove traces of chloroform. They were dried with sterilized paper filtration by the method of Atallah, 1983. Thereafter, the grains were taken for each concentration by putting 30 grains in each dish. For germination, the dishes were put in the dark. In each dish, 10 to 15 grains were put for obtaining each concentration. The dishes were incubated in the dark for seven days at 30°C, along with 2.5 ml of sterile physiological water for each dish when needed (Abrason, et al.1983; Atallah, 1983).

Treatment of seed with cytokinin

The grains of each variety were put in three phytohormone concentrations (10 mg/L, 20 mg/L, and 30 mg/L) for 1 hour, and then the grains were rinsed with distilled water.

Treatment of seed by toxins

The two varieties of chickpea were subjected to 6 concentrations of the toxin (3.5, 15, 30, 125, 250 and 500 nanograms) for 1 hour, and then were rinsed with distilled water.

Estimation of germination

Once the incubation period was over, the non-germinated seeds were counted in each dish in each concentration, and for each type of seed, the germination percentage was estimated using the following equation (Atallah, 1983).

$$\text{Germination percentage} = \frac{Total\ number\ of\ seeds - number\ of\ non-germinated\ seeds}{Total\ number\ of\ seeds} \times 100$$

Results and Discussion

Effect of Cytokinin on the germination rate of chickpea types

Based on Figure (3) which shows the average germination of the chickpeas seeds treated by cytokinin, it can be noted that the percentage of germination ranged between 89% and 97% for both varieties, and the highest percentage was recorded among FLIP 85-55 at a concentration of 20 mg/L of cytokinin. ILC 32-79 was the fastest in the germination, which accounted 96% at the same concentration of cytokinin.

The results clearly showed that the response to cytokinin varied from one species to another.So this experiment showed that soaking seeds of chickpea in cytokinin improved their germination.

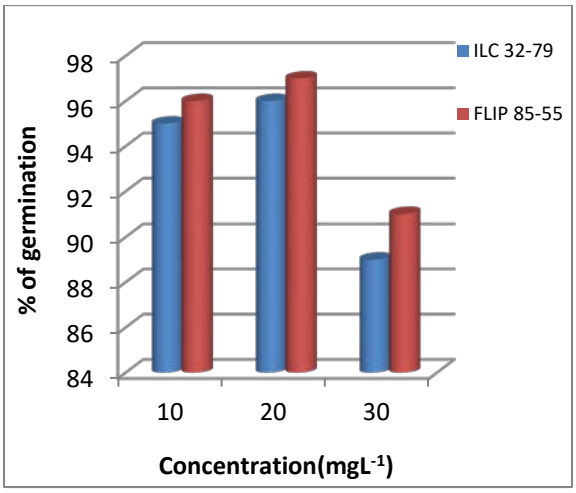

Fig. 3. Effect of Cytokinin on the germination rate.

Effect of Fungal metabolic on the germination rate of chickpea types

The recorded results in Figure (4) demonstrates the differences in the sensitivity of the two cultivars regarding the different concentrations of the toxin, the lowest rate of germination (14 %) was registered in the type FLIP 85-55 for the concentration of 500 ug, while the rate of germination in ILC 32-79 was 16 **%**. Moreover, the results showed that the variety FLIP 85-55 represented the highest degree of endurance against different concentrations of toxin.

These results were consistent with what many researchers have found. Christensen reported that the different levels of Aflatoxin B1 inhibit the germination of maize seeds (Christensen, 1973). The same was observed for seeds of chickpea (Nahdi, 1982), and for the seeds of barley (Abrason, et al. 1983). Additionally, it has been found that the lowest concentration of *Aspergillus fumigatus* toxin might affect the germination of *Triticum durum* and barley seeds (Dehimat, et al. 2010).

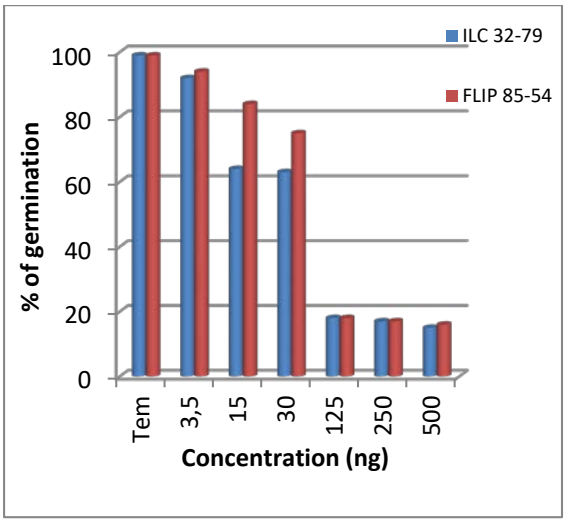

Fig. 4. Effect of Fungal metabolic on the germination rate.

Conclusion

Chickpea productivity has been often limited due to various biotic and abiotic stresses. The production of Fungal metabolic has been known to degrade the seed quality and reduce the germination. The effect of Fungal metabolic from *Aspergillus fumigatus* affected the seed germination and the seedling growth of Chickpea varieties.

With regard to the results of these experiments, it was very much clear that cytokinin is able to enhance chickpea seed germination.

Also, it seemed clear that the opposite effect on the germination of the seeds of chickpeas in the presence of secondary metabolic products by fungi can be removed to treat seeds by cytokinin.

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