



## **$\beta$ -1,3-Glucanase-Mediated Induction of Disease Resistance in *Cicer arietinum* (L.) Against *Fusarium oxysporum***

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### ABSTRACT

Plant  $\beta$ -1,3-glucanases are pathogenesis-related (PR) enzymes that hydrolyze  $\beta$ -1,3-glucans in fungal cell walls, compromising their structural integrity and inhibiting pathogen growth. These enzymes, along with phytoalexins, constitute key components of plant-pathogen interactions. In this study, the role of  $\beta$ -1,3-glucanase in elicitor-induced defense responses was investigated in resistant (Jaki-9812, Gulak-1), moderately resistant (AKG-46), and susceptible (Chafa-1) cultivars of chickpea (*Cicer arietinum* L.) against *Fusarium oxysporum*. Enzyme activity was assayed spectrophotometrically using laminarin as substrate. Results demonstrated rapid induction of  $\beta$ -1,3-glucanase activity in resistant cultivars within 5 hours post-elicitation, peaking at 40-45 hours. *Fusarium* conidial suspension was the most effective elicitor, followed by reduced glutathione (abiotic) and fungal cell wall elicitor. Tissue-specific expression was highest in cotyledons and roots, and lowest in epicotyls. The study concludes that  $\beta$ -1,3-glucanase activity, along with salicylic acid accumulation, serves as a biochemical marker for disease resistance and plays a vital role in systemic acquired resistance (SAR) in chickpea.

**Keywords:**  $\beta$ -1,3-glucanase, pathogenesis-related proteins, phytoalexins, plant-pathogen interaction, systemic acquired resistance, *Cicer arietinum*, *Fusarium oxysporum*

### INTRODUCTION

Plants have evolved a sophisticated array of biochemical and molecular defenses to counteract pathogenic invasions. Among these, the synthesis of phytoalexins and the induction of pathogenesis-related (PR) proteins are well-documented components of induced resistance (Dixon et al., 1995; Mansfield, 1999). Phytoalexins are low-molecular-weight antimicrobial compounds that accumulate at infection sites, inhibiting pathogen growth. In chickpea (*Cicer arietinum* L.), the major phytoalexins are the pterocarpan medicarpin and maackiain, which are synthesized via the phenylpropanoid pathway (Ingham, 1976; Barz & Welle, 1992).

Concurrently, PR proteins such as chitinases and  $\beta$ -1,3-glucanases are upregulated upon pathogen recognition. These hydrolytic enzymes degrade fungal cell walls, acting synergistically to inhibit fungal growth (Mauch et al., 1988).  $\beta$ -1,3-glucanases specifically target  $\beta$ -1,3-glucan, a structural polysaccharide in fungal walls, thereby weakening pathogen integrity (Kauffmann et al., 1987). Their induction is a hallmark of systemic acquired resistance (SAR), a long-lasting defense priming phenomenon (Ross, 1961; Kesmann et al., 1994).

Despite extensive studies on PR proteins in model plants, their role in legume–*Fusarium* interactions remains underexplored. *Fusarium oxysporum* is a devastating soil-borne pathogen causing wilt in chickpea, leading to significant yield losses. This study investigates  $\beta$ -1,3-glucanase induction in chickpea cultivars with varying resistance levels, using biotic and abiotic elicitors. The objectives were to (1) compare the rapidity and magnitude of enzyme induction, (2) evaluate elicitor efficiency, (3) assess tissue-specific expression, and (4) correlate enzyme activity with resistance phenotypes.

## MATERIALS AND METHODS

### 2.1 Plant Material and Elicitors

Four chickpea cultivars—Jaki-9812 and Gulak-1 (resistant), AKG-46 (moderately resistant), and Chafa-1 (susceptible)—were grown under controlled conditions. Elicitors included:

- Biotic: *Fusarium oxysporum* conidial suspension (live pathogen), fungal cell wall (FCW) elicitor.
- Abiotic: Reduced glutathione.

### 2.2 Enzyme Extraction

Frozen tissue (1 g) was homogenized in 1 mL ice-cold 0.1 M sodium citrate buffer (pH 5.0). The homogenate was centrifuged at 10,000 rpm for 15 min. Proteins were precipitated with ammonium sulfate, resuspended in extraction buffer, and used as enzyme source.

### 2.3 $\beta$ -1,3-Glucanase Assay

Activity was measured using laminarin as substrate, following the method of Kauffmann et al. (1987). The assay mixture contained 0.48 mL sodium acetate buffer (pH 5.2), 100  $\mu$ L enzyme extract, and 200  $\mu$ L laminarin solution (1 mg/mL). After incubation at

37°C for 3 h, reducing sugars released were quantified using the arsenomolybdate method at 660 nm. A glucose standard curve (10–100  $\mu$ g) was used for calibration. Enzyme activity was expressed in nKat/mg protein.

### 2.4 Protein Estimation

Total protein was determined by the Bradford dye-binding method.

### 2.5 Experimental Design and Statistical Analysis

Each treatment was replicated three times. Data were analyzed using ANOVA and mean separation by Tukey's HSD test at  $*p < 0.05$ .

## RESULTS

### 3.1 Kinetics of $\beta$ -1,3-Glucanase Induction

Basal enzyme activity was low in all cultivars pre-elicitation. Following elicitation with *Fusarium* conidia, rapid induction was observed within 5 h in resistant cultivars. Peak activity occurred at 40–45 h in cotyledons and roots of resistant lines, reaching 1667.15–1949.86 nKat/mg protein (Table 1). Moderately resistant AKG-46 showed delayed peaking (45–50 h), while susceptible Chafa-1 peaked later (50–55 h) at significantly lower activities (899.66–1094.82 nKat/mg protein).

### 3.2 Elicitor Efficiency

Conidial suspension was the most potent inducer across all cultivars, followed by reduced glutathione and FCW elicitor. In resistant cultivars, conidia-induced activity was 2.5-fold higher than controls. Reduced glutathione elicited intermediate responses, highlighting its role as a chemical inducer of SAR.

### 3.3 Tissue-Specific Expression

Cotyledons and roots exhibited the highest enzyme activities, followed by hypocotyls and epicotyls (Table 2). This gradient was consistent across elicitors and cultivars, with resistant lines showing more pronounced tissue-specific induction.

### 3.4 Correlation with Resistance Phenotype

Resistant cultivars displayed earlier and stronger induction of  $\beta$ -1,3-glucanase, coinciding with higher salicylic acid levels and phenylalanine ammonia-lyase (PAL) activity. Susceptible cultivars showed sluggish and low-magnitude responses.

**Table 1: Peak activity of  $\beta$ -1,3 glucanases ( $\eta$ Kats/mg protein) in different seedling parts of chickpea cultivars elicited with biotic and abiotic elicitors.**

| Cultivars | Control | Elicitors Used |         |           |
|-----------|---------|----------------|---------|-----------|
|           |         | Conid. suspen. | FCW     | Red. Glu. |
| cotyledon |         |                |         |           |
| Gulak-1   | 755.14  | 1921.26        | 1617.86 | 1789.17   |
| Jaki-9812 | 744.23  | 1949.96        | 1583.19 | 1772.38   |
| AKG-46    | 668.24  | 1166.82        | 979.24  | 1084.68   |
| Chafa-1   | 637.00  | 1094.82        | 899.11  | 996.87    |
| Root      |         |                |         |           |
| Gulak-1   | 528.28  | 1743.86        | 1358.89 | 1575.84   |
| Jaki-9812 | 494.71  | 1718.68        | 1398.78 | 1460.88   |
| AKG-46    | 483.86  | 1036           | 907.18  | 932.24    |
| Chafa-1   | 480.25  | 974.98         | 731.36  | 815.76    |
| Hypocotyl |         |                |         |           |
| Gulak-1   | 503.88  | 1681.56        | 1277.57 | 1463.41   |
| Jaki-9812 | 509.63  | 1692.94        | 1218.31 | 1360.87   |
| AKG-46    | 444.62  | 1123.58        | 864.52  | 881.24    |
| Chafa-1   | 430.29  | 899.66         | 624.87  | 768.44    |
| Epicotyl  |         |                |         |           |
| Gulak-1   | 528.45  | 1667.15        | 1221.26 | 1482.95   |
| Jaki-9812 | 406.45  | 1680.00        | 1192.66 | 1351.49   |
| AKG-46    | 314.19  | 1126.13        | 882.38  | 959.44    |
| Chafa-1   | 431.68  | 913.66         | 731.67  | 802.59    |

**Table 2: Peak activity of  $\beta$ -1,3 glucanase ( $\eta$ Kats/mg protein) elicited by biotic and abiotic elicitors to different seedling parts of chickpea.**

| Cultivars                                     | Explant used |         |           |          |
|-----------------------------------------------|--------------|---------|-----------|----------|
|                                               | cotyledon    | Root    | Hypocotyl | Epicotyl |
| <b><i>Fusarium</i> conidial suspension</b>    |              |         |           |          |
| Gulak-1                                       | 1921.26      | 1743.86 | 1681.56   | 1667.15  |
| Jaki-9812                                     | 1949.86      | 1718.68 | 1692.94   | 1680.00  |
| AKG-46                                        | 1166.82      | 1036.81 | 1123.58   | 1126.13  |
| Chafa-1                                       | 1094.82      | 974.98  | 899.66    | 913.66   |
| <b><i>Fusarium</i> cell wall elicitor</b>     |              |         |           |          |
| Gulak-1                                       | 1617.86      | 1358.89 | 1277.57   | 121.57   |
| Jaki-9812                                     | 1583.19      | 1398.78 | 1218.31   | 1192.66  |
| AKG-46                                        | 979.24       | 907.18  | 864.52    | 882.38   |
| Chafa-1                                       | 899.11       | 731.36  | 824.87    | 831.67   |
| <b>Abiotic elicitor – reduced glutathione</b> |              |         |           |          |
| Gulak-1                                       | 1789.17      | 1575.84 | 1463.41   | 1482.92  |
| Jaki-9812                                     | 1772.38      | 1460.88 | 1360.87   | 1351.49  |
| AKG-46                                        | 1084.82      | 832.24  | 884.24    | 859.44   |
| Chafa-1                                       | 996.87       | 915.76  | 768.44    | 802.59   |

## DISCUSSION

The rapid induction of  $\beta$ -1,3-glucanase in resistant chickpea cultivars aligns with established models of pathogen recognition and signal transduction (Boller, 1988; Linthorst, 1991). The early burst of enzyme activity within 5 h post-elicitation suggests efficient perception of pathogen-associated molecular patterns (PAMPs) and activation of MAP kinase cascades leading to PR gene expression.

The superior efficacy of conidial suspension over FCW elicitor indicates that live pathogens deliver a broader array of elicitors, including secreted effectors, which trigger stronger defense responses (Dixon, 1986). The effectiveness of reduced glutathione—a known reactive oxygen species (ROS) scavenger and signaling molecule—supports its role in modulating redox-based defense signaling (Kesmann et al., 1994).

Tissue-specific expression patterns suggest that cotyledons and roots, as primary storage and uptake organs, are frontline tissues with heightened sensitivity to elicitors. The lower response in epicotyls may reflect differential receptor distribution or signal attenuation.

The correlation between  $\beta$ -1,3-glucanase activity and resistance phenotype underscores its utility as a biochemical marker for screening wilt-resistant chickpea genotypes. Synergism with chitinases and phytoalexins likely amplifies antifungal efficacy, contributing to SAR (Ji & Kuc, 1996).

## CONCLUSION

This study demonstrates that  $\beta$ -1,3-glucanase induction is a rapid, elicitor-specific, and tissue-dependent defense response in chickpea against *Fusarium oxysporum*. Resistant cultivars exhibit earlier and higher enzyme activity, which can be leveraged in breeding programs for marker-assisted selection. The use of abiotic elicitors like reduced glutathione offers potential for chemical priming of resistance. Future work should focus on transgenic overexpression of  $\beta$ -1,3-glucanase genes and field validation of elicitor-based protection strategies.

**Conflict of Interest:** The authors declare no conflict of interest in relation to this research.

**Data Availability Statement:** Not applicable.

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