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Analysis of varietal response to bakanae infection *Fusarium fujikuroi* and gibberellic acid through morphological, anatomical and hormonal changes in three rice varieties

R.A.S. Elshafey[1*], A.M. Tahoon [2], F.A. El-Emary[3]

[1]Rice Research and Training Center, Rice Research Department, Field Crops Research Institute, Agricultural Research Center, 33717 Sakha, Kafr El-Sheikh, Egypt

[2]Rice Pathology Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt

[3]Department of Agricultural Botany, Faculty of Agriculture, Al-Azhar University (Assiut Branch), 71524 Assiut, Egypt

Abstract

***Fusarium fujikuroi*, the causal organism of bakanae disease, is mainly seed borne pathogen on rice. The response of different rice varieties have more concern to understand pathogenesis process and host pathogen interaction complex. Therefore, the present study had some objectives: to determine response of some rice genotypes to bakanae infection and Gibberellic acid (GA3) treatment through morphological, anatomical and plant hormones changes. The highly virulent isolate no. 10 of *F. Fujikuroi* was used in evaluation of three rice cultivars; Sakha 101, Giza 179 and promising line GZ 10101-5-1-1-1. Changes in all morphological, anatomical traits and plant hormones activities Gibberellic acid (GA3), Indol Acetic acid (IAA) and Abscisic Acid (ABA) with bakanae infection and GA3 treatment were assessed from 15-60 days after inoculation and GA3 treatment during season 2018. Results indicated that bakanae infection caused severe morphological changes as abnormal elongation, degradation of chlorophyll and seedling death. Morphological changes were associated with wide anatomical changes of leaf as deformation of motor cell, mesophyll layer. For stem, infection and GA3 induced significant increase in the No. of aerenchyma and their diameter and increase pith diameter, and stem elongation. As well as, anatomical changes in roots were significant increase in diameter of epidermis, cortex layers, vascular cylinder, and reduction in diameter of xylem vessels. Out of anatomical results, *Fusarium fujikuroi* prefer to grow in aerenchyma, pith, cortex, vascular bundle of both sheath and stem. There is a significant increase in plant hormones Gibberellic acid (GA3), Indol Acetic acid (IAA) and Abscisic Acid (ABA) with bakanae infection and GA3 treatment combined with bakanae infection and GA3 treatment. GZ 10101-5-1-1-1 was recorded the lowest response to GA3 treatment with the lowest infection % and stem elongation%. While Sakha 101 and Giza 179 were the highly susceptible cultivars to bakanae with the highest infection %, stem elongation% and response to GA3. The fast and highest stem elongation %, No. of nodes and internode length was considered as remarkable phenotypic markers it can be used as valuable and early selection marker of susceptibility in breeding program to bakanae disease. GZ 10101-5-1-1-1 as new promising line and high tolerant to bakanae and low response to GA3 could be used as a good source in bakanae resistance breeding program.**

Keywords: rice, *Gibberella fujikuroi*, elongation, bakanae, gibberellic acid, IAA, ABA, anatomical traits.

* **Corresponding author:** R.A.S. Elshafey,
E-mail: relshafey13@yahoo.com

Introduction

Bakanae caused by the fungal pathogen *Fusarium fujikuroi* Nirenberg [teleomorph *Gibberella fujikuroi* (Sawada) Ito & Kimura] (Carter et al., 2008; Leslie & Summerell 2006; Ou, 1985). Bakanae became more distributed on large scale with the increase of dry seeded nursery and direct seeded rice (Yang et al., 2003). Bakanae characteristic symptoms are abnormal elongation of the rice plant and foolish seedling one of the most important emerging seed and soil borne disease. In India, the yield losses ranging from 15-25 and can reached 40 % (Sunder et al., 2014; Pannu et al., 2012; Rood, 2004). Highly incidence of bakanae was demonstrated from Italy (Amatulli et al., 2012) and almost of major Asian rice growing countries such as Bangladesh, Pakistan, India, China, South Korea, Japan and Taiwan (Chen et al., 2016; Gupta et al., 2014; Park et al., 2009; Khan et al., 2000). *Fusarium fujikuroi*, the causal agent of bakanae disease, was mainly the most abundant *Fusarium* spp. (Wulff et al., 2010) that isolated from typical symptoms and only *F. fujikuroi* isolates were able to cause bakanae disease, (El-Kady et al., 2016; Amatulli et al., 2010). The fungus have ability to infect rice plants from pre-emergence to mature stages, and severe infection of rice seeds lead to poor germination (Iqbal et al., 2011). *F. fujikuroi* was hemibiotrophic fungus, their initial infection depend on living host cells as a biotrophic, and progressive infection includes consumption nutrients and host cells destruction (necrotrophic) (Ma et al., 2013). Gibberellins (GAs) are natural group of terpenoid plant hormones as secondary metabolites biomolecules complex in the fungus *Gibberella fujikuroi* with strong effects in plant physiology process. GAs was identified in some fungal and bacterial species, in some cases related to virulence (Cerezo et al., 2018; Avalos et al., 1999). Gibberellin producers belong to group C out of six groups A-F, as subspecies *fujikuroi* the pathogens of rice, with abnormal elongation as the most distinctive symptom of rice seedlings, is due to the additional growth induced by the fungal gibberellins (Klittich & Leslie, 1992; Leslie, 1991). The changes in plant morphology was contributed to the ability of *F. fujikuroi* to secrete gibberellic acids (GAs) as secondary metabolites (Ou, 1985; Bearder 1983). The fungus not depend on GAs in growth and development, as secondary metabolites although they are thought to contribute to the virulence of *F. fujikuroi*, the only Fusarium species capable of GAs biosynthesis, by controlling jasmonic acid- responsive gene expression and jasmonic acid-mediated plant immune responses (Siciliano et al. 2015; Wiemann et al. 2013). GA production was also associated with fungicide sensitivity of different F. fujikuroi isolates (Tateishi & Suga, 2015). Bioactive gibberellins play an essential role in many aspects of plant growth and development, such as stem elongation, flower and fruit devilment and seed germination (KO et al., 2006; Koga et al, 2004; Gomi & Matsuoka, 2003). The ability of fumonisins and gibberellin GA_3 production in vitro totally varied with *Fusarium* species. Whereas fumonisins were produced by most of the strains of *F. verticillioides* and *F. proliferatum*, gibberellin GA_3 was only produced by *F. fujikuroi* (Wulff et al., 2010). The only *G. fujikuroi* strains able to produce GAs and the species was determined as the pathogen of bakanae disease of rice from total 25 strains belong 5 Fusarium species (Nur Ain et al., 2008). *Gibberella fujikuroi* produce the growth hormone gibberellins, which causes plant elongation (Bhalla et al., 2010; Berrios et al., 2010). Plants produce different types of hormones, such as gibberellin (GA), auxin, abscisic acid (ABA), salicylic acid (SA), ethylene (ET), jasmonic acid (JA), cytokinin (CK), brassinosteroids (BR) and strigolactones that may play a role in plant-pathogen interactions (Bari & Jones, 2009). GA and its signaling components may play important roles in regulating defense responses against various necrotrophic and biotrophic pathogens (Bari & Jones, 2009). Based on gibberellin production, 16 strains of *Fusarium moniliforme* and one each strain of *Fusarium pallidoroseum*, *F. oxysporium* and *F. solani* observed great variability in gibberellin production from different strains of *Fusarium*. Gibberellins are responsible for the growth aberrations observed in rice plants infected with *G. fujikuroi* (bakanae disease) (Yang et al., 2013). Anatomical structure of stem was a good indicator to mechanism of GA_3 application on rice plant. Therefore, the present study had some objectives: to determine response of some rice genotypes to bakanae infection and GA_3 treatment. In addition to compare the impact of *F. fujikuroi* and GA_3 in damage of rice tissues structure through determine of morphological, anatomical and plant hormones changes.

Materials and methods

Isolation, pathogenicity and monitoring changes associated with *Fusarium fujikuroi* infection and gibberellic acid treatments: *Fusarium fujikuroi* mainly isolated from infected upper nodes of stem just above crown of diseased plants of different rice cultivars. Small pieces of upper infected nodes and internodes 1-3 cm were cut and washed under tap water, sterilized with 3% NaOCl for 2-3 min, rinsed twice in sterile distillate water. One-week later growing mycelia transferred to fresh PDA media in Petri plates and kept at room temperature. All isolated Fusarium isolates were tested in their reaction and virulence on different rice cultivars. For proper identification *F. Fujikuroi*, only isolates of *F. Fujikuroi* that mainly pathogenic to rice and able to induce typical symptoms of bakanae disease (Wulff et al., 2010). The isolated fungus was identified according to Leslie and Summerell (2006), Nelson et al. (1983) and Booth (1971). Preliminary trail was conducted to determine the proper concentration of GA_3 whereas five concentrations at 10, 20, 40, 80 and 160 ppm were added to the nutrients solution. In addition 15 rice genotypes were used to select the current tested genotypes and Minghu 63 was replaced by Giza 179 as a local cultivar that has the same response to infection and GA_3 treatment. Then, the highly virulent isolate no. 10 of *F. Fujikuroi* was used in inoculation of different rice cultivars. Fungal culture sub-cultured in PDA at room temperature. One week later, fungus mass grown in Petri dishes scraped in sterile water with a spatula. The final suspension was filtered through two layers of sterile cotton lint. Grains of Sakha 101 the most susceptible cultivar, Giza 179, Minghu 63 indica japonica and GZ 10101-5-1-1-1 as highly tolerant promising line, were soaked in spore suspension of the tested isolate at concentration 5×10^5 spores / ml for 48h. Then, all inoculated grains were incubated for additional two days. All treatments were arranged as healthy, infected check and 80 ppm concentration of GA_3 with three replicates for each cultivars in complete randomize design. Fifty grains of each cultivar was seeded in hydroponic method in nutrient solution in 10 x 10 cm. diameter plastic pots and grown in the greenhouse at 30-35°C. All morphological changes as, total shoot and root lengths measured, plant height, leaf length, chlorophyll content, pathological traits combined with bakanae infection, such as elongation %, adventitious roots, and bakanae infection were recorded. The elongation % was calculated according this formula:

Elongation (%) with GA_3 = (GA_3 treatment – Control)/Control×100.

Elongation (%) with bakanae infection = (Infected treatment – Control)/Control×100.

Extraction, separation and estimation of growth regulating substances: The all parts of rice samples were collected 42nd day after sowing, immediately dipped in liquid nitrogen with 0.5 gm fresh weigh for each treatment and stored at frozen conditions. The extraction method was analyzed at Food Technology Research Institute, Agricultural Research Centre according to Shindy and Smith (1975) and Hassanein et al. (2009) as following; to estimate the amounts of acidic hormones IAA, ABA and GA_3, the plant hormone fractions and standard ones were methylated according to Vogel (1975) to be ready for GC analysis. Flame ionization detector was used for identification and determination of acidic hormones using Helwett Packered Gas Chromatography (5890). The chromatography was fitted and equipped with HP-130 mx 0.32 mm x 0.25 mm capillary column coated with methyl silicone. The column oven temperature was programmed at 10°C/min from 200°C (5 min) to 260°C and kept finally to 10 min. Injector and detector temperature were 260 and 300°C, respectively. Gases flow rates were 30, 30, 300 cm/sec for N2, H2 and air, respectively and flow rate inside column was adjusted at 2 ml/min. Standards of IAA, GA_3 and ABA were used. Peak identification was performed by comparing the relative retention time of each peak with those of IAA, GA_3 and ABA standards. Peak area was measured by triangulation and the relative properties of the individual components were therefore obtained at various retention times of samples. Elongation percentage and rice plant hormones Gibberellic acid (GAs), Indol-3-acetic acid (I.A.A) and Abscisic acid (A.B.A) were estimated 30 days after sowing and the fungus inoculation. The number of dead seedlings was counted 30 days after sowing and inoculation.

Anatomical studies: To investigate the effect of bakanae disease infection and GA_3 treatment on some anatomical changes in structure of leaf, stem, and roots of rice cultivars;, Sakha 101, Giza 179 and GZ 10101-5-1-1-1. This investigation was carried out at laboratory and greenhouse of Rice Research Center, Sakha, Kafr El-Sheikh, Egypt and laboratory of Botany department, Faculty of agriculture, Assuit, El- Azhar University, Assiut, Egypt during rice season 2018 . The samples were taken from all genotypes after 15 and 60 days after sowing and seed inoculation of all treatments to study anatomical structure of leaf, stem and root by 40 x magnifying. Ten samples of the tip portion of second leaf, 0.5 cm after second stem node and 0.5 from the tip of root were collected of each treatment. Each sample measured 0.5 cm. All samples were killed and fixed for 48 hours in FAA (10 ml. formalin, 5 ml glacial acetic acid, 50 ml ethyl alcohol and 35 ml water). The dehydrated samples were infiltrated and embedded in paraffin (52-54°C m.p.). The embedded samples were sectioned on a rotary microtome at a thickness of 5-10 µm. Sections were mounted on slides and deparaffinized. Staining was accomplished with safranine and light green, cleared in xylol and mounted in Canada balsam (Gerlach, 1977). Slides were microscopically examined and measurements and counts. The sections were computerized morphometrical analysis, the morphometrical analysis was done by Research Microscope type Axiostar plus made by Zeiss transmitted light bright field examinations upgrade able to professional digital image analysis system (Carl Zeiss Axiovision Product Suite DVD 30).

Statistical analysis: Statistical analysis conducted using Costat computer statistical software package, Data were statistically analyzed according to the analysis of variance (ANOVA) of the completely randomized design, applied in both laboratory and greenhouse experiments according to Gomez and Gomez (1984). Least Significant Difference (LSD) test at 5% was used to determine genotypic differences among all means of pathological, morphological and anatomical traits under each treatment.

Results and Discussion

Monitoring of pathological and morphological changes associated with bakanae infection: Concerning morphological changes, invasion of *Fusarium fujikuroi* to rice tissues, fungus induced significant changes in infected rice cultivars compared with healthy one. The earliest symptoms appeared fifteen days after inoculation, as an inoculated rice seedling exhibited abnormal elongation and during this stage, infected seedling produced early nodes and very long internodes as characteristic symptoms to highly sensitive cultivars to bakanae infection and high response degree to gibberellic acid (GA_3) treatment. Infected seedling became taller, slender and highly chlorotic than healthy adjacent seedlings. The hyperelongation of bakanae infected plants due to secretion of *F. fujikuroi* to Gibberellin and plant hormones. The infected seedlings were thin with yellowish to pale green leaves (Figure 1a). Tillering ability, the infected seedlings completely lose their tillering ability and produce individual plant during whole season. The healthy seedling bear two tillers with the main culm compared with single infected one (Figure 1b). With longitudinal section of infected stem (Figure 1b), the internal surface of upper nodes had dark brown color and covered with fungal mycelium mass than white healthy nodes, therefore, *Fusarium fujikuroi*’s recovery percentage increased from lower nodes to higher-level nodes, in agreement of Manandhar (1999). In response of main roots infection, from lower to upper nodes production of adventitious roots in progress as the most characteristic symptoms and specific response to *Fusarium fujikuroi* severe infection (Figure 1c, d). The infected leaves became pale yellow and chlorotic with remarkable degradation of chlorophyll as chlorosis appearance. In addition, leaves were severely rolled and converted to thin slender tubes reflected a wide reduction in leaf area. Therefore, all physiological processes will be affected as a result of chlorophyll damage and leaf area reduction (Figure 1c, d). The most evident typical symptoms of Bakanae are slender thin stem, severe yellow chlorotic and abnormal elongated leaves and seedlings that induced as a result of pathogen production of gibberellin (Amatulli et al., 2010; Amoah et al., 1995; Ou, 1987).

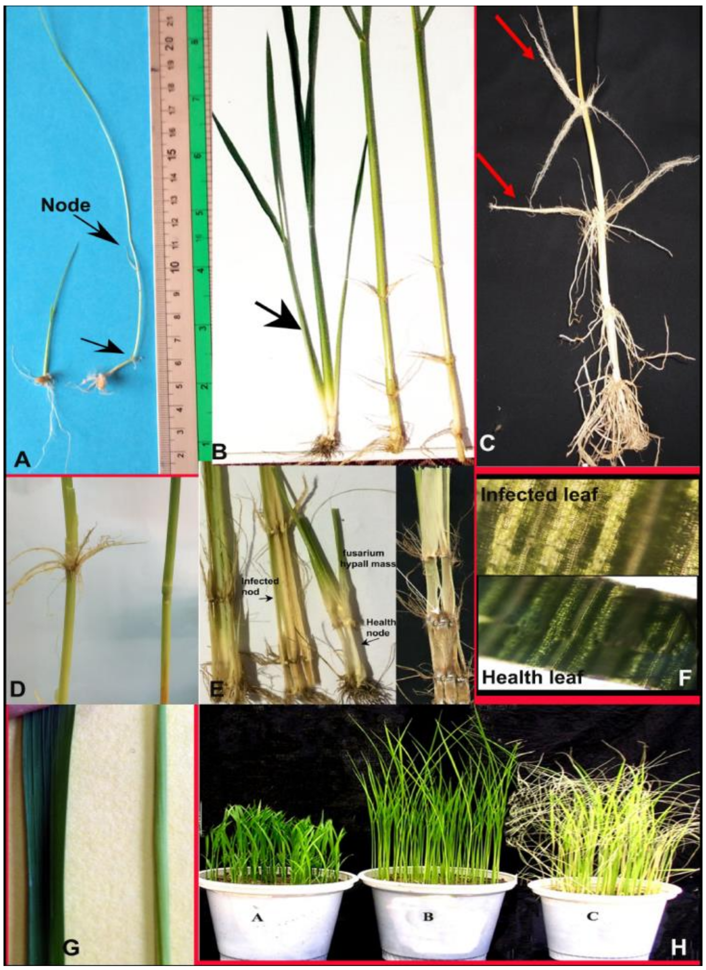

Figure 1: Morphological changes combined with infection of *F. fujikuroi* A: abnormal elongation. B: tillering ability, C and D: adventitious roots, E: Hyphal growth inside node, F: chlorophyll degradation and chlorosis., G: leaf rolling, H: healthy, infected and highly infected seedlings.

We selected rice cultivars with divergent sensitivity to bakanae infection for this study, Data in Table (1) revealed that there are significant difference in response to bakanae infection among rice varieties. Sakha 101 as highly susceptible cultivars that exhibited the highest level of infection percentage

58.33 % followed by Giza 179 which gave 53.33. Whereas, GZ 10101-5-1-1-1 recorded the lowest infection 38.33. Both cultivars Sakha 101 and Giza 179 reflected high level of susceptible response to *F. fujikuroi* infection, in contrary, GZ 10101-5-1-1-1 recorded high level of tolerance to infection in comparison to Sakha 101. For elongation percentage as a good indicator to response degree to bakanae infection, elongation percentage was in parallel with level of infection and highly associated with bakanae infection and GA_3 treatment. Data in Table (2) and Figure (3) indicated that Giza 179 was superior in their response to infection of bakanae under artificial inoculation that gave 50 % over the healthy check. While, Sakha 101 as a semi dwarf cultivar showed the second rank of highest elongation percentage 46.43. Whereas, GZ 10101-5-1-1-1 as new promising line have the lowest elongation 20 % taller over than the check. The increase of GA_3 was found associated with elongation of internodes and chlorosis of leaves in susceptible plants of MR 211 (Quazi et al., 2015). For response to GA_3 treatment, GA_3 treatments were surpassed infection of bakanae in their effect of treatment that reflected in abnormal elongation. Giza 179 was highly responsive to GA_3 with the highest elongation percentage, followed by Sakah 101, while GZ 10101-5-1-1-1 has the lowest response to GA_3 with 27% compared with 60-62 %. This results in agreement with Rangaswamy (2012), and Kwon and Paek (2016) they reported that gibberellic acid is a plant growth regulator which produce different effects comprising stem elongation, enzyme induction, leaf and fruit senescence, growth regulation, seed germination and flowering. The number of nodes and length of internode 21 days after sowing, as a good indictor to susceptibility to bakanae and GA_3, Sakha 101 and Giza 179 recorded both the highest no. of nodes and internode length nodes within 3 weeks . out of our results, those both traits was more associated, fast, more progressive and specific with infection of bakanae and susceptibility of cultivar than GA_3 treatment response. Therefore, Sakha 101 as highly susceptible cultivar with high infection developed more nodes in short time with the highest length. Then, no. of nodes and length of internode were good selection traits for breeding program to bakanae disease as an early selection marker of susceptibility.

Table 1: Pathological and morphological changes in rice varieties associated with bakanae infection and GA_3 treatment.

Variety	Treatment	Infection (%)	Shoot length1 5DAI (cm)	Root length1 5DAI (cm)	Root Reduction (%)	Stem Elongation (%)	No. of nods	Length of internode 21DAS (cm)	Dead plants 21DAS (%)	Chlorophyll content (SPAD)	Leaf length (cm)	Droopy plants % with abnormal elongation	Time after inoculation to start drop (Day)
Sakha 101	Healthy	0.00	15.33	13.33		-	0	0.00	0	35.33	6.67	0.00	0.00
	Infected	58.33	28.30	5.67	57.46	46.43	2.33	6.70	56.67	23.00	25.67	11.67	30.00
	80 ppm GA_3	0.00	38.33	3.67	72.47	62.50	2	2.33	51.67	21.33	29.67	45.00	25.00
Giza 179	Healthy	0.00	15.33	10.31		-	0	0.00	0	32.67	7.33	0.00	0.00
	Infected	53.33	28.33	7.30	29.19	50.00	1	8.70	53.33	21.33	30.33	91.67	25.00
	80 ppm GA_3	0.00	38.33	4.33	81.92	60.53	2	6.50	58.33	21.00	35.33	96.00	15.00
GZ101 01-5-1-1-1	Healthy	0.00	23.67	8.35		-	0	0.00	0	34.33	7.33	0.00	0.00
	Infected	38.33	29.33	6.32	24.31	20.00	0	0.00	11.67	27.67	17.67	1.00	60.00
	80 ppm GA_3	0.00	32.67	3.33	60.12	27.27	1	3.00	8.33	25.33	23.33	11.67	60.00
L.S.D 5%		2.885	1.842	1.013			0.4561	0.645	4.997	1.099	1.033	3.251	1.033

DAI = day after inoculation, DAS = day after sowing.

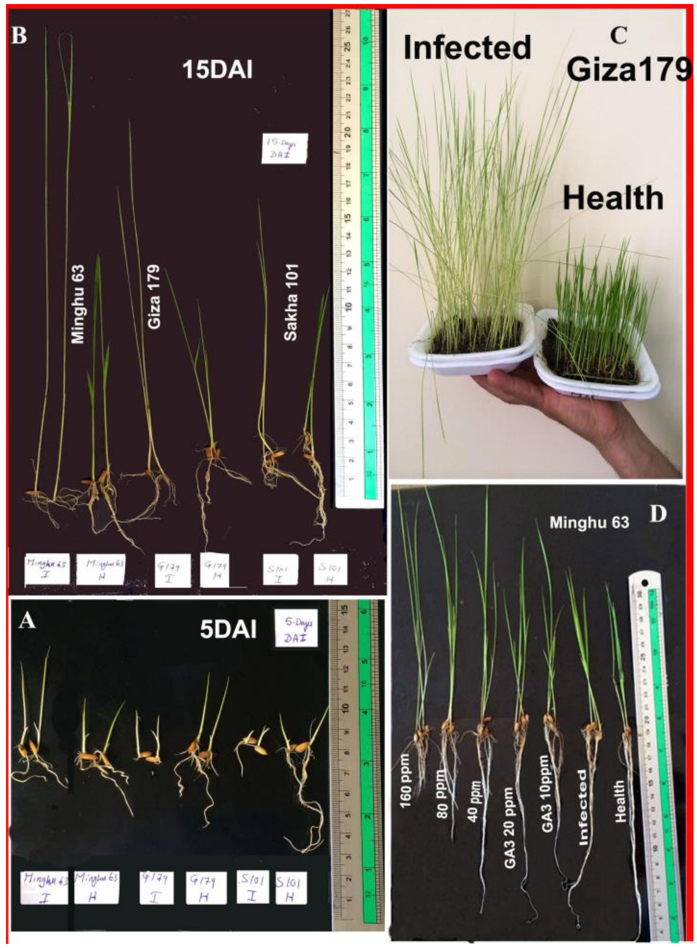

Figure 2: A: Infection with *G. fujikuroi* and their effect on rice plant elongation percentage, shoot and root 5 DAI (day after inoculation), B: 15 DAI, C: healthy and highly infected plants, D: different level of GA_3.

These results in agreement with Hwang et al. (2013) revealed that *F. fujikuroi* produced gibberellic acid (GA_3) which is involved in plant growth regulation. GAs accumulate within and around rice roots during host recognition, pre-penetration morphogenesis, and pathogen growth in the plants, and GAs are believed to be responsible for abnormal internode elongation of stem because high concentrations of this hormone cause hypertrophy of the cells in the parts of rice found above ground. In extreme infection, infected plants fall over and die. The response to GA_3 treatment was more evident with Giza 179 from percentage of droopy plants whereas more than 95 % of tested plants were started to droop, collapse and lodged after 15 days from treatment date compared with GZ 10101-5-1-1-1 that gave almost 12 % lodging after 60 days as very late response (Table 1 and Figure 3). These results in harmony with Quazi et al. (2015) reported that with severe symptoms progression of bakanae infection and increase of GA_3, after 21 days of inoculation, susceptible plants of MR 211 were started to collapse and lodged due to over elongation.

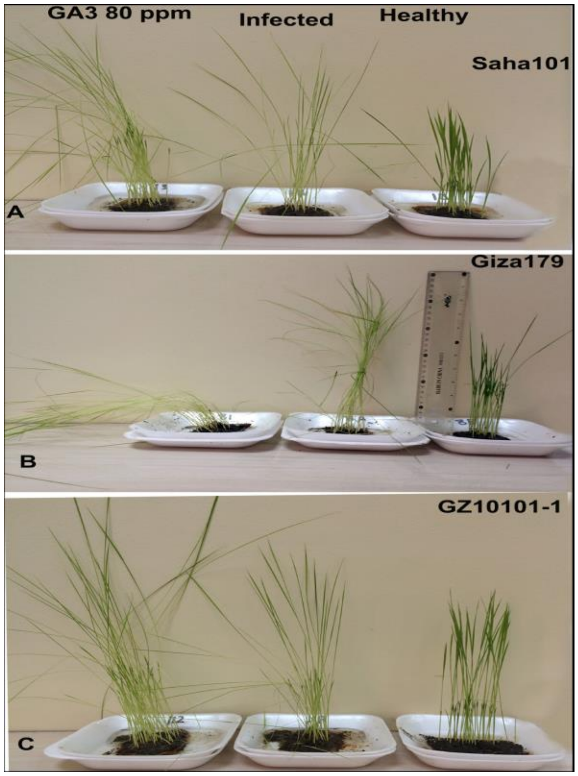

Figure 3: Effect of infection of *G. fujikuroi* and GA_3 treatment 80 ppm on rice plant elongation percentage and droopy plants of rice cultivars Sakha 101, Giza 179 and GZ 10101-5-1-1-1.

The damage and deleterious effect of both bakanae infection and GA_3 was clear in reduction of root length that ranged with bakanae infection from 24.31 to 57.46 and GA_3 treatment caused remarkable reduction ranged from 60.12 to 81.92 % (Table 1, Figure 1a, Figure 2 a, b and d). In addition, the chlorophyll content of GZ 10101-5-1-1-1 more stable for long time and their content not affected more than infected plants, whereas healthy plants have 34.33 SPAD and infected plants 27.67 and GA_3 treatment 25.33 with low significant differences. The low degradation of chlorophyll and chlorosis was a good indicator for tolerance of GZ 10101-5-1-1-1 to bakanae infection. These results are in agreement with Hwang et al. (2013) who reported that isolate FfB20 of *F. fujikuroi* triggered rice seedling elongation and foolish growth. In addition, symptoms on rice roots inoculated with FfB14 or FfB20 isolates, drastically inhibited root growth, and the roots and crowns were decayed. In trial on various concentrations and effect of GA_3 on Minghu 63 with 10, 20, 40, 80 and 160 ppm, GA_3 exhibited the same behavior and effect of *F. fujikuroi* that induced the reduction of root length and induction of abnormal elongation with increase of GA_3 concentration (Figure 3d). GA_3 concentration at 80 ppm recorded the same effect of 160 ppm, so we used 80 ppm in study of anatomical changes. Gibberellins are responsible for the growth aberrations observed in rice plants infected with *G. fujikuroi* (Yang et al., 2013). According to pathological and morphological response to bakanae infection and GA_3, GZ 10101-5-1-1-1 exhibited more desirable traits for bakanae tolerance as late response to GA_3, low infection of bakanae, more stable chlorophyll content. Finally, we can recommend, according our results, using this line as a high tolerance resource in a successful bakanae disease breeding program.

Table 2: Anatomical changes in leaf of rice cultivars associated with bakanae infection and GA_3 treatment.

Variety	Treatment	Leaf thickness (µ)	Mesophyll (µ)	Upper epidermis (µ)	Motor cell length (µ)	Motor cell width (µ)	Bundle sheath (µ)	Bundle Ø (µ)	M.xylem Ø (µ)	Midrib length (µ)	Midrib width (µ)
Sakha 101	Healthy	95.64	70.98	14.25	53.54	81.8	27.22	102.06	31.78	169.00	147.89
	Infected	65.46	31.95	24.64	25.70	42.1	22.74	114.78	22.74	140.54	114.45
	80 ppm GA_3	69.26	36.25	21.59	26.66	41.5	29.70	101.35	19.75	135.65	139.65
Giza 179	Healthy	102.86	71.24	18.48	52.43	74.2	39.27	176.47	27.19	170.38	160.87
	Infected	82.77	52.63	20.61	24.30	50.1	26.35	115.93	16.9	110.56	128.76
	80 ppm GA_3	71.57	45.54	15.17	26.47	37.6	16.40	93.85	14.03	125.78	131.95
GZ101 01-5-1-1-1	Healthy	119.24	95.00	16.65	51.51	80.6	50.65	118.9	25.64	172.89	170.34
	Infected	105.32	79.93	13.51	48.57	72.7	44.19	120.72	22.12	166.15	164.81
	80 ppm GA_3	100.67	81.99	14.15	48.91	67.4	38.59	111.49	17.2	156.79	156.37
L.S.D 5%		4.433	5.933	3.642	3.723	11.28	3.587	4.537	3.328	6.264	4.632

Leaf anatomical changes associated with bakanae infection and GA_3 treatment: Concerning the effect of Bakanae disease infection on leaf anatomy, F. fujikuroi infection induced a significant change in the anatomical structure of the third leaf of rice seedlings at 21 DAS (Figure 4 and Table 2). Data revealed that some tissues were decreased, while the others increased in thickness or diameter as a clear response to the fungus infection. The third leaf thickness, mesophyll, bundle sheath thickness and midrib width, in the infected plants were reflected highly significant reduction in opposite to healthy plants. For leaf damage, some anatomical changes reflected morphological damage due to infection such as reduction in leaf area and rolling. Figure 4a and b illustrated that the fungus infection caused a sever reduction in leaf area and rolling compared with full stretched healthy leaves (Figure 4c). Regarding leaf thickness, both susceptible cultivar Sakha 101 and Giza 179 recorded highly significant reduction compared with tolerant one GZ 10101-5-1-1-1. Whereas, thickness of infected Sakha 101 leaves was 65.46 µ compared to healthy 95.64 µ on the other hand GZ 10101-5-1-1-1 was 105.32 µ opposite to 119.24 µ, (Table 2 and Figure 4F, G). Therefore, tolerance of GZ 10101-5-1-1-1 was clear evident from many traits such as, stable leaf thickness. The same trend of reduction was recorded with different tissues; Mesophyll layer, bundle sheath thickness and midrib.

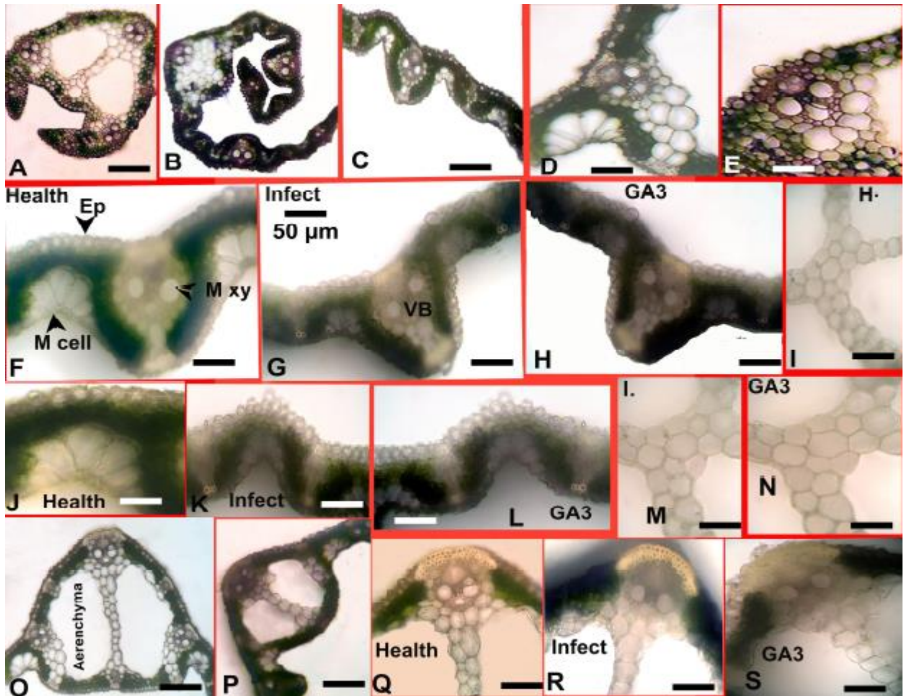

Figure 4: Transverse section of rice leaf and anatomimcal structure due to *G, fujikuroi* Infection. A: infected rice leaf 15 DAI. B: Rolling of infected leaf 15 DAI. C: Health leaf. D and E: health and infected Midrib region. F, G and H: health, infected and GA_3 treatment. I, M and N: health, infected and GA_3 paranchyma cell of midrib. J, K and L: health, infected, GA_3 motor cell and epidermal cell. O and P: health and infected midrib. Q, R and S: VB of midrib. Bar=50µ.

For mesophyll layer thickness, it was sharply decreased by more than two times compared with the healthy leaves, while, infected plants of Sakha 101 gave 31.95 compared to 70.98 µ. On the other hand, GZ 10101-5-1-1-1 exhibited low reduction in Mesophyll layer thickness. The reduction in a Mesophyll thickness was highly associated with sharp reduction in chlorophyll content and chlorosis, so it should significantly affect photosynthesis process and subsequently reduces grain yield of infected plants. Therefore, this explained that GZ 10101-5-1-1-1 still have high chlorophyll content after bakanae infection compared with both Sakha 101 and Giza 179. For GA_3 treatment, GA_3 exhibited the same trend and impact of reduction on all aforementioned traits. Subsequently, GA_3 have the same behavior of *F. fujikuroi* infection in anatomical changes of leaf. For motor cells, both dimensions of motor cell was sharply decreased with bakanae infection and GA_3 treatment, more than 50 % in both Sakha 101 and Giza 179, in the opposite direction motor cell of GZ 10101-5-1-1-1 not affected. So, the leaf rolling and deformation as a result of bakanae infection and GA_3 treatment was highly associated with damage of motor cells, subsequently the lamina be rolled (Table 2 and Figure 4B, J, K, L). Out of our results, all leaf anatomical changes with infection and GA_3 treatment was highly associated with and morphological changes, and reflected damage in all leaf tissues. The response of xylem and vessel diameter to infection was clear in reduction with almost 40-50 % of Sakha 101 and Giza 179 with infection and GA_3 treatment. The damage of xylem vessel was sharply and negatively affected water absorption, water content, nutrients and starch translocations. Also, the rest of anatomical traits such as bundle sheath μ, bundle Ø, and midrib dimensions μ were decreased in response to infection and GA_3 treatment (Table 2 and Figure 4, F-H, I, M, N, O-S). Only upper epidermis layer exhibited an increase in its cells diameter response to infection and GA_3 opposite behavior to all other anatomical leaf traits (Table 2 and Figure 4, F-H). So, deformation of leaf structure due to bakanae infection and GA_3 treatment, all growth process and nutrients translocation will be affected. The deformation of leaf structure actually will be associated with yield loss. Anatomical structure of flag leaf of susceptible rice cultivars significantly changed in response to bakanae infection and GA_3 treatments. All morphological and anatomical changes in leaf structure of rice were matched with the same changes that caused by white tip nematode infection. These findings are in accordance with Artyukhova and Popova (1981) and Elshafey et al. (2010) who recorded ultrastructural changes caused by *A. besseyi* to leaves of susceptible rice plants. They reported that nematode causes crimping and chlorosis, considerable changes in the lamina, the structure of the epidermis, and misalignment, underdevelopment and deformation of the motor cells. Also, these results are in harmony with findings of Jairajpuri and Baqri (1991), who reported that the injury due to the infection of *A. besseyi* caused by the stylet leads to the disintegration of phloem cells. It is clear that white tip nematode disease deforms and damages the anatomical structure of rice flag leaf, which is the important organ for photosynthesis. *Aphelenchoides besseyi* caused a sharply decrease in the chlorophyll content of the cell, so the photosynthesis rate is severely and negatively affected. According to the deformation of flag leaf due to infection of white tip nematode all agronomic traits will be decreased. White tip nematode infection deformed the motor cells which controlled the rolling and expanding of leaf blade, so the leaf area which exposed to the light of sun sharply diminished as a result of this damage the photosynthesis rate reached to the lower level. Vascular bundle, xylem diameter were negatively affected with white tip nematode infection as a result of this damage the translocation of nutrients from source to the sink will be affected. As a result of all above-mentioned damages, the growth of the plant will be affected, these findings in accordance with results of Tahoon (2016) reported that bakanae infection caused a reduction and loss in chlorophyll content, leaf area, abnormal elongation and produce small panicles with high sterility. The bakanae infection and GA_3 decreased most of leaf anatomical characters. The tolerant and susceptible genotypes revealed wide variation and differentiating parameters in leaf anatomy.

Sheath anatomical changes associated with bakanae infection and GA_3 treatment: Bakanae disease infection induced significant changes in the anatomical structure of sheath of rice cultivars. Figure (5) illustrated that tissues of highly infected cultivar Sakha 101 reached to senescence after 21 days from inoculation, this was clear in Figure (5a) with sheath of health plants compared with infected ones in Figure (5B, C), red arrows in Figure (5C) refers to accumulation of unknown substances in infected cells. In addition, air space or Aerenchyma of infected sheath (Figure 5D) was full occupied with *Fusarium* hyphal mass compared with health tissues (Figure 5H). Whereas, the health tissues of sheath can reached the senescence conditions within 60 days after sowing (Figure 5F, G).Therefore, infection of *Fusarium fujikuroi* induced senescence of infected tissues in short period of time than healthy leaves and blocking of aerenchyma with growth of fungus reduced the amount of available air and respiration process. The growth of fungus inside tissues affected all growth process and consume nutrients. Consequently, the yield was negatively affected with the infection of bakanae disease. The fungus significantly induced abnormal elongation of sheath cells (Figure 5K) compared with health cells Figure (5I, J). These findings are in agreement with Hwang et al. (2013) found that *F. fujikouri* vigorously infected rice stems.

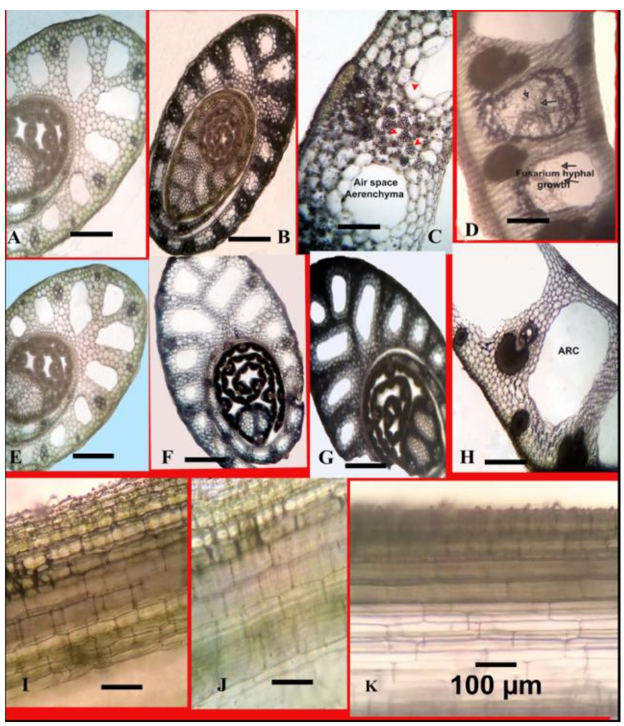

Figure 5: Transverse section of rice sheath and anatomical structure due to *F. fujikuroi* Infection. A: health rice leaf inside sheath 21 DAI. B: infected sheath and senescence 21 DAI, sheath. C: accumulation of unknown substances inside infected cells 21 DAI. D: hyphal growth in Aerenchyma (ARC), H: healthy and free ARC. E: Healthy sheath. F, G: accumulation of unknown substances inside healthy cells 60 DAS. I: longitudinal section of health sheath. J: infected leaf. K: GA_3 treated sheath, bar 100µ.

Stem anatomical changes associated with bakanae infection and GA_3 treatment: Data presented in Table (3) and Figure 6 showed that, bakanae infection and GA_3 treatment, in the present study increased the most varieties stem anatomical features compared with health plants. The significant increase was evident in the no. of aerenchyma and their diameter. Whereas, the number of aerenchyma increased from 0 in health plants to 14 in highly susceptible of Sakha 101 and 7 aerenchyma with Giza 179, while only one air space (aerenchyma) was formed in infected stem of tolerant variety GZ 10101-5-1-1-1. In addition, there is significant increment in diameter of this aerenchyma with infection and GA_3 treatment. Aerenchyma (Gas spaces) forms as an adaptation to submergence to facilitate gas exchange. In rice (*Oryza sativa*), aerenchyma develop by cell death and Lysis, and H_2O_2 promotes aerenchyma formation in a dose-dependent manner (Steffens et al., 2010). Cell death in response to biotic or abiotic stresses is often mediated by plant hormones. In addition, reactive oxygen species (ROS) superoxide anion radical (O_2^-) and hydrogen peroxide (H_2O_2) are central regulators of plant cell death (Bouchez et al., 2007; Overmyer et al., 2003; Moeder et al., 2002). The infection of *Fusarium* and GA_3 treatment may be induced Lysis and cell death of stem cell, free radicals release such as O_2^- and H_2O_2 therefore, increase the aerenchyma formation. This fungus was aerobic and prefers aeration for production of GA_3 and this aeration condition surely necessary because the route of the biosynthesis of GAs involves a series of oxidative steps. Therefore, the microorganism's demand for oxygen may increase with the growth of mycelium. Oxidative steps, which are catalyzed by citocromo P450 monooxygenases, dioxygenases and dehydrogenases, a high aeration condition is critical for an optimal production process (Tudzynski, 2005; Machado et al. 2001; Tudzynski, 1999). Therefore, for more aerobic conditions *F. fujikuroi* demand wide aerenchyma number and were available in highly susceptible cultivars than resistant.

Table 3: Anatomical changes in stem tissues of rice cultivars associated with bakanae infection and GA_3 treatment.

Variety	Treatment	No. of aerenchyma	Aerenchyma* Ø (μ)	Vascular bundle Ø (μ)	No. of Vascular bundle	M. xylem Ø (μ)	Pith Ø (μ)	Ground tissues Ø (μ)	Stem Ø (μ)	Stem cell length** (μ)	Stem cell elongation (%)
Sakha 101	Healthy	0.00	0.0	139.8	29.33	23.0	110.20	41.40	1649.30	23.72	-
	Infected	14.33	186.0	142.8	29.67	30.0	634.60	66.50	1660.00	44.20	46.33
	GA_3 80 ppm	9.33	223.0	233.8	11.00	69.0	271.50	97.10	2008.30	58.96	59.77
Giza 179	Healthy	0.00	0.0	141.8	29.67	22.0	107.60	38.40	1641.70	17.97	-
	Infected	7.33	187.0	161.8	13.67	40.0	428.50	65.40	1216.30	38.56	53.40
	GA_3 80 ppm	7.33	197.0	194.4	13.67	66.0	518.80	124.10	1683.80	46.09	61.01
GZ 10101-5-1-1-1	Healthy	0.00	0.0	145.1	30.00	23.0	109.80	38.70	1655.10	16.36	-
	Infected	1.00	108.0	299.8	12.67	66.0	417.70	89.10	2338.80	30.21	45.85
	GA_3 80 ppm	1.00	158.0	384.7	12.00	116.0	461.50	185.80	2838.70	31.03	47.28
L.S.D 5%		0.807	100.7	28.49	0.950	7.5	22.20	12.58	21.21	6.634	

* diameter of average no. of aerenchyma, ** longitudinal section of stem.

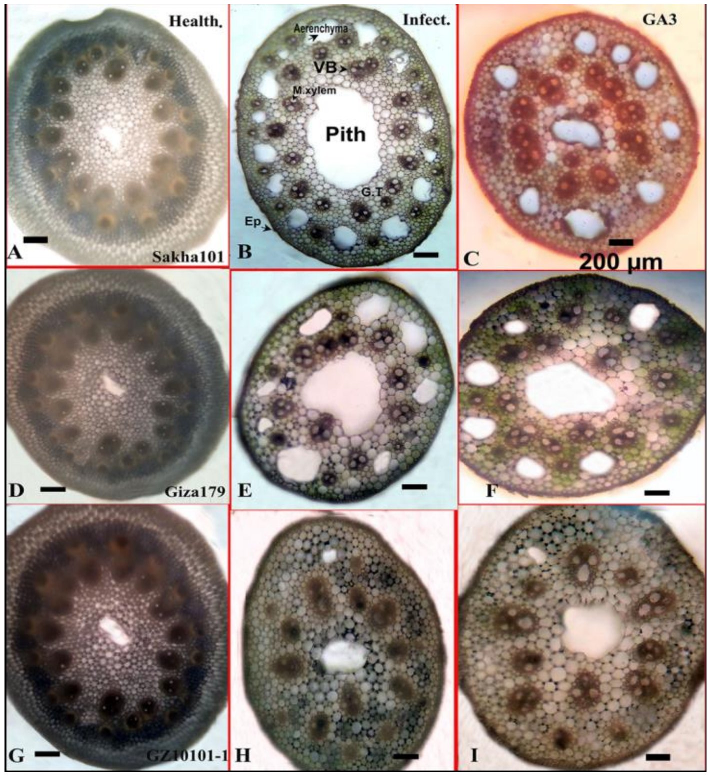

Figure 6: Transverse section of rice stem and anatomical structure due to *F. fujikuroi* infection and GA_3 80 ppm treatment. A: health stem of Sakha 101. B: infected stem of Sakha 101. C: GA_3 treated stem of Sakha 101. D, E, F: health, infected and GA_3 treatment of Giza 179. G, H, I: health, infected and GA_3 treatment of GZ10101-5-1-1-1, bar 200µ. VB: Vascular bundle.

The anatomical features that increased with infection and GA_3 treatment were cleared in Vascular bundle Ø, M. xylem Ø, Pith Ø µ, Ground tissues Ø, and Stem cell elongation %. The invasion of *Fusarium fujikuroi* to vascular bundle and surrounded tissues of GT induced a significant increase in diameter of M. xylem and this may be contributed to feeding of this fungus on cell wall and secretion of different cell lytic enzymes such as cellulose increase weakness and breaking down of cell wall. Therefore, it caused easy expanded and stretched of cell wall of infected cells and consequently increased in diameter (Table 3 and Figure 7). our results illustrated that the *Fusarium* fungus have a wide preference in their growth to occupy pith area and progress in growth through the direction to GT cells and finally vascular bundles (Figure 7B-F). Our results in line with Hwang et al. (2013) who reported that Invasive mycelial growth of isolates FfB14 and FfB20 in the rice stem harvested at 7 dpi. FfB14 vigorously infected rice stems, and active invasive mycelial growth was observed. FfB20 also grew in the rice stem; however, the growth was relatively lower. In addition revealed the active fungal growth of FfB14 in the root and crown of rice seedlings, while the growth rate of FfB20 in rice was more than 4 times lower than FfB14. Massive infective mycelial growth of FfB14 was evident in rice stems and crown; however, FfB20 did not exhibit vigorous growth. The role of GA_3 was more marked and evident in stem cell elongation % and it was clear that this fungus have the ability to induce production of GA_3, therefore infection of Fusarium exhibited almost the same level of elongation of internodes. In addition, the same trend of cell response with infection of *Fusarium* to all stem tissues and effect of GA_3 indicated that GA_3 paly an essential role in infection process and both virulence and susceptibility of cultivars. The tested varieties exhibited a wide variation in their response to both infection and GA_3. The tolerant variety GZ 10101-5-1-1-1 reflected various responses than highly susceptible cultivar Sakha 101, whereas, GZ 10101-5-1-1-1 recorded the highest increment in all stem anatomical features specially Vascular bundle Ø, M. xylem Ø, Ground tissues, low stem elongation % and this increment in side of bakanae tolerance (Table 3 and Figure 6).

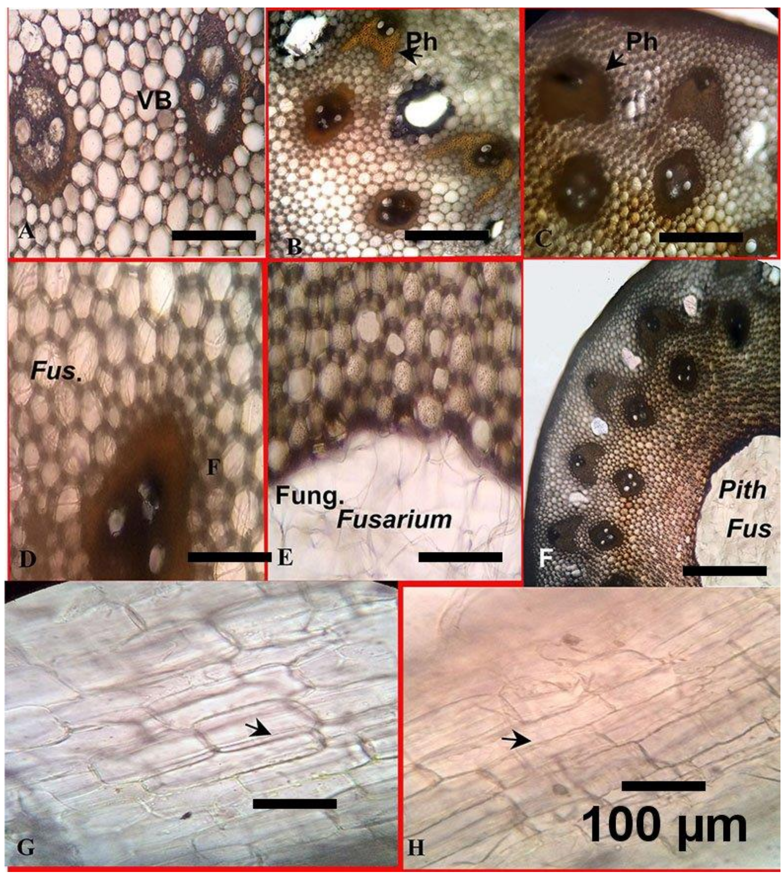

Figure 7: Transverse section of rice stem and anatomical structure due to *G. fujikuroi* infection. A: health stem. B, C, D: invasion and development of infection in vascular bundle. E, F: growth of *Fusarium fujikuroi* in pith and cortex cells. G: longitudinal sections of health stem cell. H: longitudinal sections of infected and abnormal elongated cell, bar 100µ. VB: Vascular bundle, ph: phloem.

GZ 10101-5-1-1-1 was recorded the lowest response to GA_3 treatment, whereas the sensitivity to GA_3 combined with highest stem elongation % as with Giza 179 as 60 % compared with 47.28 % of GZ 10101-5-1-1-1. The high sensitivity to GA_3 associated with high level of bakanae susceptibility. The fast and highest stem elongation % was considered a remarkable phenotypic marker it can be used as valuable selection marker in breeding program to bakanae disease.

Root anatomical changes associated with bakanae infection and GA_3 treatment: Data presented in Table (4) and Figure (8) showed that, bakanae infection and GA_3 treatment, in the present study increased the most varieties root anatomical features compared with health plants except M. xylem vessels Ø. The increment in diameter or cell expansion have a fixed direction from epidermal cell to cortex until reached to vascular cylinder. Therefore, the hypertrophy response of for-mentioned root tissues caused a stress and pressure on M. xylem vessels; consequently, the diameter of M. xylem will be reduced and blocked. The reduction in M. xylem diameter will be associated with deficit of water absorption resulted in low water content and wilt.

Table 4: Anatomical changes in root tissues of rice cultivars associated with bakanae infection and GA_3 treatment.

Variety	Treatment	Epidermal thickness (μ)	Cortex thickness (μ)	Cortex cell diameter Ø (μ)	Vascular cylinder Ø (μ)	M. xylem vessels Ø (μ)
Sakha 101	Healthy	28.84	138.69	18.79	111.27	15.87
	Infected	60.60	159.74	42.29	136.02	7.51
	GA_3 80 ppm	58.00	165.03	47.10	164.80	7.14
Giza 179	Healthy	30.97	134.50	17.53	142.95	11.25
	Infected	54.70	150.70	39.44	169.12	7.56
	GA_3 80 ppm	53.66	158.73	43.80	178.89	7.01
GZ 10101-5-1-1-1	Healthy	29.33	137.48	17.15	146.85	11.43
	Infected	44.90	145.64	32.18	158.24	8.39
	GA_3 80 ppm	51.78	151.91	41.24	168.22	7.24
L.S.D 5%		4.971	6.031	4.120	4.302	2.295

The infection of *Fusarium fujikuroi* to root tissues caused collapse, breakage of cell wall, degradation and finally cell Lysis and death as clear in Figure (8F). Finally, Sakha 101 and Giza 179 exhibited the highest response in all root anatomical traits compared with GZ 10101-5-1-1-1 and healthy check. Data in Table (5) represented the correlation among infection of bakanae disease, GA_3 treatment, morphological, anatomical, plant hormones traits of rice verities. Data indicated that there a highly significant and positively correlation among infection of bakanae infection and some anatomical features such as elongation percentage, number of aerenchyma, aerenchyma Ø, stem cell length, stem cell elongation, epidermal thickness, cortex thickness (μ), cortex cell diameter Ø, stem vascular cylinder Ø (μ), stem M. xylem vessels Ø, GA_3. Whereas, highly significant and negatively correlated with chlorophyll content, leaf thickness (μ), motor cell length (μ), motor cell width (μ), leaf bundle sheath (μ), bundle Ø, leaf M. xylem Ø, midrib length (μ), number of stem vascular bundle, stem M. xylem vessels Ø (μ), root M. xylem vessels Ø.

Table 5: Correlation coefficients among infection of *F. fujikuroi*, GA_3 treatment and some morphological, anatomical, plant hormones traits of rice verities.

	Infection	Elongation	Chlorophyll	GA3	IAA	ABA
Infection (%)	1	0.966**	-0.981**	0.743*	0.669*	0.327
Elongation (%)	0.966**	1	-0.970**	0.808**	0.540	0.341
Chlorophyll content	-0.981**	-0.970**	1	-0.745*	-0.637	-0.416
Leaf length (cm)	0.975**	0.975**	-0.983**	0.838**	0.539	0.384
Leaf thickness (μ)	-0.805**	-0.865**	0.788*	-0.659	-0.504	-0.214
Mesophyll (μ)	-0.746*	-0.821**	0.741*	-0.586	-0.524	-0.238
Upper epidermis (μ)	0.383	0.471	-0.437	0.057	0.534	0.350
Motor cell length (μ)	-0.878**	-0.940**	0.894**	-0.731*	-0.598	-0.492
Motor cell width (μ)	-0.918**	-0.965**	0.925**	-0.789*	-0.530	-0.282
bundle sheath (μ)	-0.622	-0.687*	0.602	-0.711*	-0.279	-0.247
bundle Ø	-0.530	-0.536	0.428	-0.521	-0.125	-0.050
M.xylem Ø	-0.842**	-0.809**	0.879**	-0.777*	-0.384	-0.352
midrib length (μ)	-0.829**	-0.893**	0.881**	-0.783*	-0.505	-0.675*
midrib width (μ)	-0.713*	-0.752*	0.704*	-0.541	-0.589	-0.427
No. of aerenchyma	0.798**	0.829**	-0.780*	0.496	0.686*	0.306
Aerenchyma Ø	0.984**	0.967**	-0.985**	0.697*	0.635	0.346
Vascular bundle	0.310	0.162	-0.292	0.045	0.132	-0.170
No. of Vascular bundle	-0.693*	-0.650	0.710*	-0.586	-0.286	-0.265
M. xylem Ø	0.509	0.409	-0.505	0.330	0.116	-0.122
Pith Ø	0.853**	0.715*	-0.803**	0.494	0.780*	0.296
Ground tissues (μ)	0.559	0.461	-0.552	0.423	0.117	-0.127
Stem Ø	0.098	-0.052	-0.051	-0.173	-0.009	-0.477
Stem cell length (μ)	0.903**	0.951**	-0.883**	0.692*	0.498	0.172
Stem cell elongation	0.977**	0.925**	-0.964**	0.709*	0.638	0.330
Epidermal thickness (μ)	0.970**	0.929**	-0.966**	0.592	0.735*	0.350
Cortex thickness (μ)	0.938**	0.947**	-0.909**	0.666	0.538	0.125
Cortex cell diameter Ø (μ)	0.973**	0.944**	-0.965**	0.677*	0.602	0.257
Vascular cylinder Ø (μ)	0.671*	0.654	-0.732*	0.686*	0.213	0.272
M.Xylem vessels Ø (μ)	-0.872**	-0.808**	0.900**	-0.565	-0.619	-0.280
GA_3	0.743*	0.808**	-0.745*	1	0.102	0.195
IAA	0.669*	0.540	-0.637	0.102	1	0.521
ABA	0.327	0.341	-0.416	0.195	0.521	1

Gibberellic acid activity changes with bakanae infection and GA_3 treatment: Data presented in Table (5) indicated that, there was a remarkable increase in GA_3 (ppm) associate with infection of bakanae and GA_3 treatment in comparison with healthy plants. For correlation coefficients, GA_3 was gave highly significant and positively correlation infection (%), infection (%), elongation (%), leaf length (cm), upper epidermis (μ), aerenchyma Ø, stem cell length (μ), stem cell elongation, cortex cell diameter Ø (μ), root vascular cylinder Ø, while was highly significant and negatively correlated with chlorophyll content, motor cell length (μ), motor cell width (μ), bundle sheath (μ), M.xylem Ø, midrib length (μ), midrib length (μ) (Table 5).

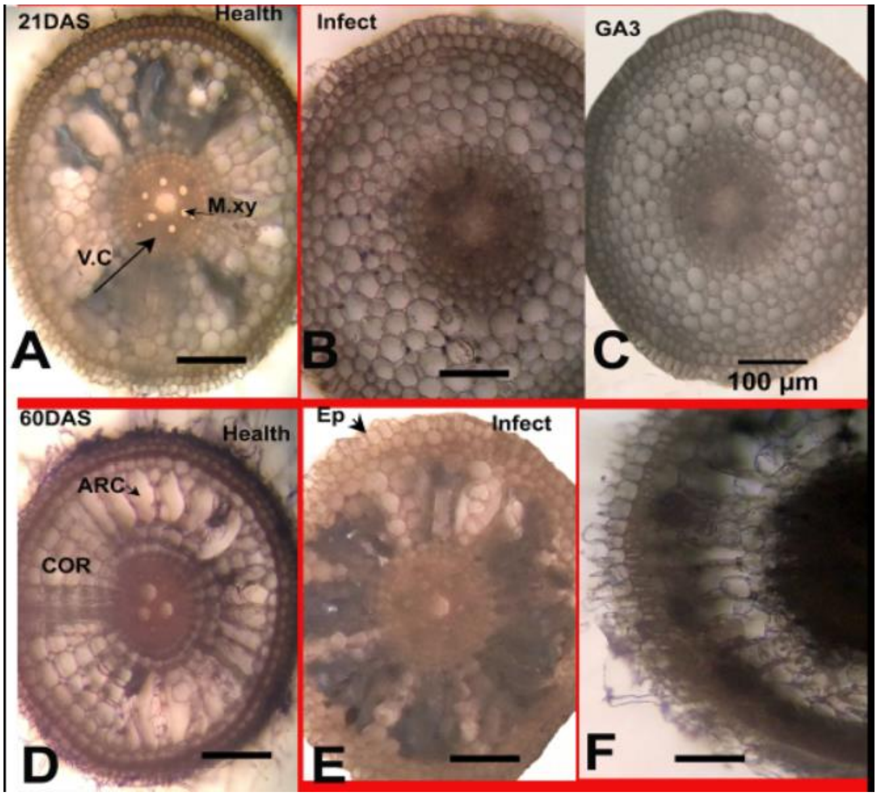

Figure 8: Transverse section of rice root and anatomical structure due to *G. fujikuroi* infection. A: health root of Sakha 101, 21 DAI. B: infected with bakanae. C: treated with 80 ppm GA_3. D: health section 60 DAI. E: infected section. F: highly damaged roots. Bar= 100 μ, Ep, epidermal cells. Co: Cortex. V.C.: vascular cylinder. X.V: xylem vessels. ARC: Aerenchyma. M.xy: Meta xylem.

Indol acetic acid activity changes with bakanae infection and GA_3 treatment: For the changes in activity of indol acetic acid activity (IAA), data presented in Table (5) indicated that, there was increasing in IAA (ppm) under all infection percentage compared with healthy plants in all rice cultivars under the present study. For correlation coefficients, IAA was gave highly significant and positively correlation infection (%), elongation (%), leaf length (cm), upper epidermis (μ), aerenchyma Ø, stem cell length (μ), stem cell elongation, cortex cell diameter Ø (μ), root vascular cylinder Ø, while was highly significant and negatively correlated with chlorophyll content, motor cell length (μ), motor cell width (μ), bundle sheath (μ), M. xylem Ø, midrib length (μ), midrib length (μ) (Table 5).

Table 6: Plant hormones changes through rice cultivars associated with bakanae infection and GA_3 treatment.

Treatment	Plant hormones in rice plants (ppm)								
	Sakha 101			Giza179			GZ 10101-5-1-1-1		
	GA	IAA	ABA	GA	IAA	ABA	GA	IAA	ABA
Healthy plants	350	0.8	0.7	310	2.5	0.5	300	0.7	0.7
Infected plants	500	20	2.3	850	15	10.5	420	15	1.7
GA3 (80 ppm)	850	8.2	0.8	1520	5	0.8	510	6.5	0.9

Abscisic acid activity changes with bakanae infection and GA_3 treatment: Concerning the abscisic acid (ABA) (ppm) data in Table (5) cleared that there was positively impact on ABA (ppm) with increase infection of *F. fujikuroi* in the present study, were its led to increase it compared with healthy plants in the tree cultivars under the present study. Abscisic acid probably compromises rice defense against pathogens. Jiang et al. (2010) demonstrated that ABA suppresses the basal resistance of rice when it interacts with the pathogen Pyricularia oryzae. For the situation and relationship among different hormones, Elemary et al. (2015) demonstrated that abscisic acid (ABA) was higher in the untreated entries or varieties with GA_3. The highest value of ABA was observed with untreated IR69625A. For Indol acetic acid (IAA) showed highly significant and positive correlation with plant height, amylose content% and GA_3, While, abscisic acid showed significant and negative correlation with plant height, number of panicles per plant, seed set %, grain yield per plant all results in agreement with Kim et al (2014) reported that, ABA able to improve abiotic stress tolerance in rice, fine regulation of its expression will be required to avoid deleterious effects on agricultural traits. In addition**,** Quazi et al. (2015) found that the increase in GAs including fungi produced GA3 and IAA were higher in inoculated susceptible MR 211 rice plants (GAs = 26%, IAA = 40.39%) as compared to resistant BR3 (GAs = 19%, IAA = 4.27%), 7 days after inoculation. 14 days after inoculation both phytohormones were observed to increase., after 21 days of inoculation with abnormal elongation, and finally dead followed by a decrease in GAs and IAA but an increase in ABA. In resistant variety BR3, marginal up regulation of GAs and IAA were observed only at 21 days after inoculation in stems with no typical symptom of bakanae disease. The increase of infection combined with remarkable increase of GA levels with both cultivars Sakha 101, Giza 179 and GZ 10101-5-1-1-1. In addition, GA3 increase with infection of bakanae disease and treatment of GA3. Infection of bakanae induced increase of IAA and ABA. While, GA_3 application was more associated with increase of IAA than ABA (Table 6). All results in agreement with Tahoon (2016) who reported that correlation coefficient values indicated that there are significantly and positively correlation among infection % and elongation %, GA, IAA and ABA. Also, elongation percentage was positively correlated with concentration of GA and both IAA and ABA. GA content positively correlated with both IAA and ABA in both Sakha 101 and Giza 179 cultivars. Considering the synergistic relationship between GA_3 and IAA, and antagonistic relationship between GA3 and ABA (Chen et al., 2006; Xu et al., 1998), it was postulated that GA 3, IAA and ABA phytohormones might be involved in different bakanae symptoms expression. As endogenous GA 3 levels were found to be correlated with elongation (hypertrophy) in bakanae diseased plants (Kuo & Yang. 1967), therefore, the justification of the current study was that the up-regulation level of GA_3 might also be responsible for the up- and down- regulation of IAA and ABA and thereby associated with other symptoms expression. Moreover, in relation to bakanae disease development, IAA and ABA that might have influences on disease susceptibility/ resistance and have not been identified. Conversely, the concentration of abscisic acid remained constant in the resistant cultivar; however, the levels of this phytohormone were highest in the inoculated plants compared to the uninoculated control (Bolwell et al., 2002; Blee et al., 2001). Accumulation of ethylene and GA and a decreased ABA level in the rice internode thus favor induction of epidermal cell death and ensure that Programmed cell death (PCD) is initiated as an early response that precedes adventitious root growth. PCD further promoted by gibberellin (GA). Gibberellic acid was also shown to promote ethylene-induced cell death, while abscisic acid (ABA) acts as a strong repressor of the epidermal cell death response (Steffens & Sauter, 2005; 2009). In conclusion, symptoms of bakanae disease including plant height increase, inhibiting chlorophyll formation, disruption of root growth and susceptibility to infection are associated with phytohormonal imbalance of GAs, IAA and ABA (Quiz et al., 2015). All pathological, morphological, anatomical traits and hormonal activities changes due to bakanae infection and GA_3 treatment were highly matched and reflected the same behavior. GZ 10101-5-1-1-1 as a new promising line was highly tolerant variety that can be used a good donor for bakanae resistance in breeding program.

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