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# EVALUATION OF VARIETAL RESISTANCE AND PHYSIOLOGICAL CHARACTERS OF FALSE AND KERNEL SMUT DISEASES OF RICE IN EGYPT

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## ABSTRACT

Rice false and kernel smuts are worldwide diseases present almost in rice ecosystems. Smut diseases are new production constrains. In Egypt, these smuts were considered a minor disease. False smut infects some grains of the panicle causing a yield loss. In addition, it produces ustiloxin which is a poisonous toxin to humans and other animals. Kernel smut also reduces the yield and quality of grains. The present study aimed to determine the resistance of number of rice genotypes against both false and kernel smut. Also study the physiological characters of the isolated fungi. Twenty three Egyptian rice genotypes were used in this investigation under field conditions in Sakha experimental farm during the 2009 and 2010 rice growing seasons. In case of false smut, all tested varieties were susceptible with different level of susceptibility as Giza 171, Giza 172, Giza 177 and Giza 178 were highly susceptible although Giza 171 and Giza 172 as late mature and Giza 177 is an early maturing variety. Data presented here show a wide range of variability in the response of different rice types against false smut infection. The resistance level of Japonica type against the disease was ranged from 20.37 to 92.90 %. While, the resistance level of Indica rice was ranged from 68.15 to 83.21 %. Also hybrid rice showed similar Indica rices behavior whereas their resistance level ranged from 66.82 to 81.88 %. In case of Indica- Japonica rice resistance were ranged from 54.01 to 88.06 %. The resistance of GZ lines ranged from 67.68 to 92.04 %. The widely cultivated varieties, Sakha 101 and Sakha 104 showed to be more resistant than other cultivars and exhibit significant a degrees of field resistance to false smut (92.90 %) compared with highly susceptible cultivar Giza 171. Regarding to kernel smut, Giza 171, Giza 172, Giza 159, Giza 177 and Giza 178 were the most susceptible tested cultivars. While Sakha 101, Sakha 104, GZ6522, GZ7955, Giza 182 and the Egyptian Yasmine were moderately resistance cultivars presenting over 90 % disease recovery. The physiological studied showed that false smut was grown abundantly on rice bran agar medium with fairly sporulation, while potato sucrose agar showed to be the most suitable medium for growing kernel smut fungus. The isolates of both fungi exhibited different level of variability. False smut isolates were differed in their colors with a range from olive to white or orange, while kernel smut isolates differed in pigments production with a range from violet or purple up to reddish color.

**Keywords :** False smut , Kernel smut , *Ustilaginoidea virens, Tilletia barclayana*, Chlamydospores, teliospores.

## INTRODUCTION

Rice false smut of rice caused by hemibiotrophic *Ustilaginoidea virens* (Cke.) Tak. (teleomorph *Villosiclava virens*) (Tanaka *et al*. 2008; White *et al*. 1990) has long been considered as a minor disease (Ou, 1985), but recently it has become a serious problem in some rice growing areas including Asia, Africa, the United States, South America, Italy, (Ou 1985; Bischoff *et al*. 2004; Ashizawa *et al*., 2005). The disease was recognized

carefully in Arkansas in 1997 (Cartwright *et al*., 1998) and is it now presents in many rice-producing countries (Cartwright *et al*., 1999). Prior to the heading stage, the fungus invades rice spikelets and cover the grains of the panicle by powdery, dark olive green spores during maturation (Ou, 1985). The fungus may invade any part of the panicle, resulting a yield loss. In addition, it produces the toxin ustiloxin which is known to be poisonous to humans and other animals (Koiso *et al*.,1992). The disease was recorded in Egypt for the first time on the old commercial rice cv. Giza 171 in Menshlien village, Kallien district, Kafr El-sheikh governorate in 1997, (Sehly *et al*., 1999). By the year 2000 the disease spread out to cover other rice growing governorates i.e. Behiera, Gharbia, Sharkia, Dakahlia, and Damietta, (Sehly *et al*., 2001), on both early and late maturing cultivars with different degrees of infection severity. (Sehly *et al*., 2000, Tahoon, 2005 and RRTC, 2009). The fungus transforms individual grains of the panicle into greenish spore balls of a velvety appearance which start small but visible in between the glumes, by the time they gradually grow to reach 1 cm or more in diameters, enfolding the floral parts. Then they start to be slightly flattened, smooth and yellow, are covered by a membrane. The membrane bursts as the result of further growth and the color of the ball become orange and later yellowish-green or greenish-black, (Ou, 1985). Although this disease does not significantly affects the rice yield and seed quality, it remains an important disease due to the production of ustiloxin toxin, which is a microtubule inhibitor toxic to humans and animals (Luduena *et al*., 1994; Koiso *et al*., 1994).

Kernel smut disease caused by *Tilletia* barclayana (Bref.) Sacc. & Syd., The genus *Tilletia* Tul. & C. Tul. comprises ca. 140 species restricted to hosts in the grass family (Poaceae) and is the largest genus in order Tilletiales (Basidiomycota, Ustilaginomycetes, Exobasidiomycetidae) (Vánky 2002). *Tilletia* is characterized by the formation of pigmented teliospores. Rice kernel smut is widespread and presents almost in all different rice ecosystems allover the world (Ou, 1985). The disease started to cause problem in some locations of Egypt viz: Belkas, Dakahlia governorate, which have wider cultivated areas of Giza 178 rice cultivar. Kernel smut was considering a minor disease. It has found in upland or irrigated rice growing countries. Today, it spread throughout Asia, Australasia, Europe, Central America, South America, North America, and Africa (Fischer and Holton, 1957; Ou, 1985; CMI, 1999; Webster and Gunnell, 1992; Chahal, 2001 and Biswas, 2003). Teliospores of *T. barclayana* are released from smutted kernels before or during harvesting and contaminate the soil. After planting, teliospores float on the surface of paddy water, germinate, and produce primary and secondary sporidia, which are forcibly discharged into the air and carried onto rice panicles. These sporidia infect ovaries through open florets and cause smutted kernels, (Whitney, 1989). The soilborne teliospores of the fungus serve as the primary source of inoculum for the initiation of this disease. The disease causes losses in both yield and quality of harvested grains. Yield losses occur from completely smutted grains lost in harvesting operations. Quality losses come from partially smutted grain which, when milled, turn entire lots of milled rice to gray color from spores liberated during milling process (Whitney, 1989 and Gravois and Bernhardt, 2000). Cultivars

are known to vary in their susceptibility to kernel smut (Cartwright *et al*., 2001 and Tsuda *et al*., 2006).

The objective of this research is designed to evaluate the varietal resistance to both false and kernel smuts as well as to study physiological characters of the isolated fungi from the contaminated areas in Egypt.

## MATERIALS AND METHODS

**Isolation and identification of the causal agent:**

False smut isolation, samples of false smutted rice grains exhibiting typical symptoms of the disease were collected from different rice governorates of Egypt. Infected grains (fresh pseudosclerotia) were carefully washed with tap water and cut into small pieces. Surface sterilized in 5% sodium hypochlorite solution for three minutes, then washed thoroughly in sterile distilled water and dried carefully between sterilized filter papers. The fungal masses were transferred onto PDA media in petri dishes and incubated at 28°c for ten days. Pure colonies were transferred onto rice bran dextrose agar medium (200 g Rice bran, 20 g Dextrose, 20 g Agar, in one liter of tap water) and incubated for 15 days to obtain pure culture (Singh *et al.* 1987). The isolated fungus was identified following Verma and Singh, 1988 and Wang *et al*. 1998).

For Kernel smut isolation, severely smutted panicles of different rice cultivars were collected from fields at the end of season. The smutted spikelets containing mass of mature teliospores of *T. barclayana* were surface-sterilized in 5% sodium hypochlorite solution for 2-3 minutes and washed twice in sterilized water. The teliospores were then soaked in sterilized water and the spore suspension was incubated overnight at 28 °C to stimulate germination. After incubation, the teliospores were centrifuged at 3000 rpm for 3 min. and the pellet was resuspended in 5 ml of 0.5% sodium hypochlorite. The teliospores then washed twice by removing the supernatant and the pellet resuspended in 1 ml sterile distilled water then add in series dilution. The pellet of each dilution was spread onto water agar supplemented with streptomycin at conc. of 0.005%. After 14 days, the germinated teliospores were transferred to PDA medium again to maintain the pure cultures of the causal organism. The cultures were incubated on both solid and liquid nutrient media.

**Effect of different media on growth characters**:

Five natural media were used in this investigation, viz : Potato dextrose agar medium (PDA) (200g potato, 20g dextrose, 20 agar/ 1L water), Potato sucrose agar (PSA), (200g potato, 20g dextrose, 20g sucrose), Rice bran agar (RBA), (200g rice bran, 20g glucose, 20 agar), Banana dextrose agar (BDA), (200g banana, 20g glucose, 20g agar), Oat meal agar (OMA), (200g oat, 20g glucose, 20g agar), Rice grains flour agar media (RFA) 200g rice flour, 20g glucose, 20g agar. Petri dishes containing the different media were inoculated with discs of 6 mm diameters of false or kernel smut isolate. Three replicates of each isolate were incubated at 25-28°C for two weeks. The radial growth was then measured. The fully grown cultures were kept for one more week for fungal sporulation. For harvesting the spores, cultures in

dishes were flooded with sterilized water (10ml/dish) and gently scraped. Spore suspension density were determined as number of spores/ml using haemocytometer. Fresh and dry weight of each isolate was determined in liquid broth of the same different previous media. Approximately three plugs from a fresh growing culture were transferred to 100 ml of different media contained in 250 ml Erlenmeyer flasks and grown at 250C for 14 days in an orbital shaker (120 rpm). Fresh mycelium was harvested by filtration through No. 3 Whitman filter paper. After drying at 50° C, the dry weight was recorded.

**Varietal Resistance:**

Two field experiments were conducted at the experimental farm of Rice Research and Training Center (RRTC), Sakha, Kafr EL-Sheikh during 2009 and 2010 rice growing seasons to evaluate the resistance level of different rice cultivars to both false and kernel smut diseases. Twenty three Egyptain rice genotypes were used (as shown in Table, 1). The experiment was carried out in a Randomized Complete Block Design (RCBD) with three replications. Thirty day old seedlings of each genotype individually transplanted in seven rows/plot/replicate with spacing of 20 x 20 cm a part between rows and plants. The nitrogen fertilizer was added as Urea (46 % N) according to the recommendation package of each cultivar. Two thirds of nitrogen dose was incorporated to top 15cm of the dry soil as a basal application before transplanting, while one third of nitrogen dose was added thirty days after transplanting. Other cultural practices were undertaken as recommended. All cultivars were inoculated three times during flowering stage at the period of florets opening (11-1 pm) with kernel smut sporidial suspension of 2 × 105 sporidia/ ml (allantoid sporidia). Gelatin was added to the inoculum at a concentration of 2.5 g/L (Sehly *et al*., 2009) to enhance the adhesion of spores on florets surfaces. Samples were taken at the end of season.

Natural infection of false smut was recorded on the rice varieties under field conditions. While kernel smut, rice varieties were evaluated under both natural and artificial inoculations. The incidence of false smut and kernel smut were tested after complete heading during the seasons of 2009 and 2010. Both smuts were reported as number of smutted grains/ panicle of each variety.

## RESULTS AND DISCUSSION

**Isolation and identification of the causal organisms:**

Spores of false smut, were slightly flattened, smooth and yellow, and covered by a membrane, Figure (1). In the field, the fungus partially transforms individual grains of the panicle into greenish spore balls which is commonly known as false smut balls of a velvety appearance during maturity, the surface of which are covered by powdery dark-olive spores (chlamydospores) as described by (Ou, 1985).

Late on, the membrane bursts showing balls orange to yellowish-green or olive-green in color, Fig (2). At this stage, the surface of the ball cracks. Chlamydospores formed on the spore balls are borne laterally on

minute streigmata on radial hyphae which spherical to elliptical, warty, olivaceous. Chlamydospores germinate in culture by germ-tubes producing septate mycelia which are ready to form conidiophores bearing conidia at the tapering apex. These conidia showed to be ovoid, very minute and germinate to produce secondary conidia. These conidia are holoblastically and sympodially produced at the apex of each conidiophore cells, Fig (1).

The chlamydospores over the winter in the soil and become a primary source of infection of the rice plants. The chlamydospores germinate on coleoptile epidermal cells of rice seedlings, and infection hyphae invade intercellular spaces and reach the meristematic tissues of rice plants (Ikegami 1963). At the booting stage, the fungus invade rice spikelets and infect rice florets these data is in agreement with (Ou, 1985, Ashizawa and Kataoka 2005; Zhou *et al*. 2003).

Spore germination occurred typically as described by Hashioka *et al*., (1951) who reported that Chlamydospores germinated in water to produce fine germ tubes bearing 1-3 conidia, Fig (1). Also it was found that more growth of germ tubes and a greater number of conidia took place in sugar solution than in water. While some researchers believe that primary infections are initiated mainly by ascospores produced from the sclerotia, under Egyptian conditions, the perfect stage and ascospores as a primary inoculum not discovered yet, so in Egypt Chlamydospores still play a vital role in primary and secondary infection which are major parts of the disease cycle.

Spores of kernel smut, were grown on potato sucrose agar media (PSA) the germinated teliospore emerged germ-tubes and formed a promycelium. This promycelium did not septate. but from 12 - 24 filamentous basidiospores formed at the tip of the promycelium, Fig. (1). The basidiospores germinate and produce filliform and allantoid sporidia (Crescent-shaped conidia) generally bear from mycelium on short streigmata. The filliform and allantoid sporidia were discharged from strigmata and germinated and produced mycelium which able to infect the florets tissues, ovary and produced smut balls of teliospores, Fig. (1). The obtained results are in accordance with those of (Durán, 1987; Vánky and Bauer, 1992; Vánky and Bauer, 1995; Goates, 1996; Castlebury and Carris, 1999).

Allantoid sporidia were germinated directly via germ tubes to produce mucelium or to produce additional sporidia, (Fig. 1). Filiform sporidia were formed from short, lateral sporogenous cells on the hyphae. Allantoid sporidia showed to be primary infective agents of *T. horrida*, (Fig. 1). This results are in agreement with the funding of (Ingold, 1996 and Ingold,1997).

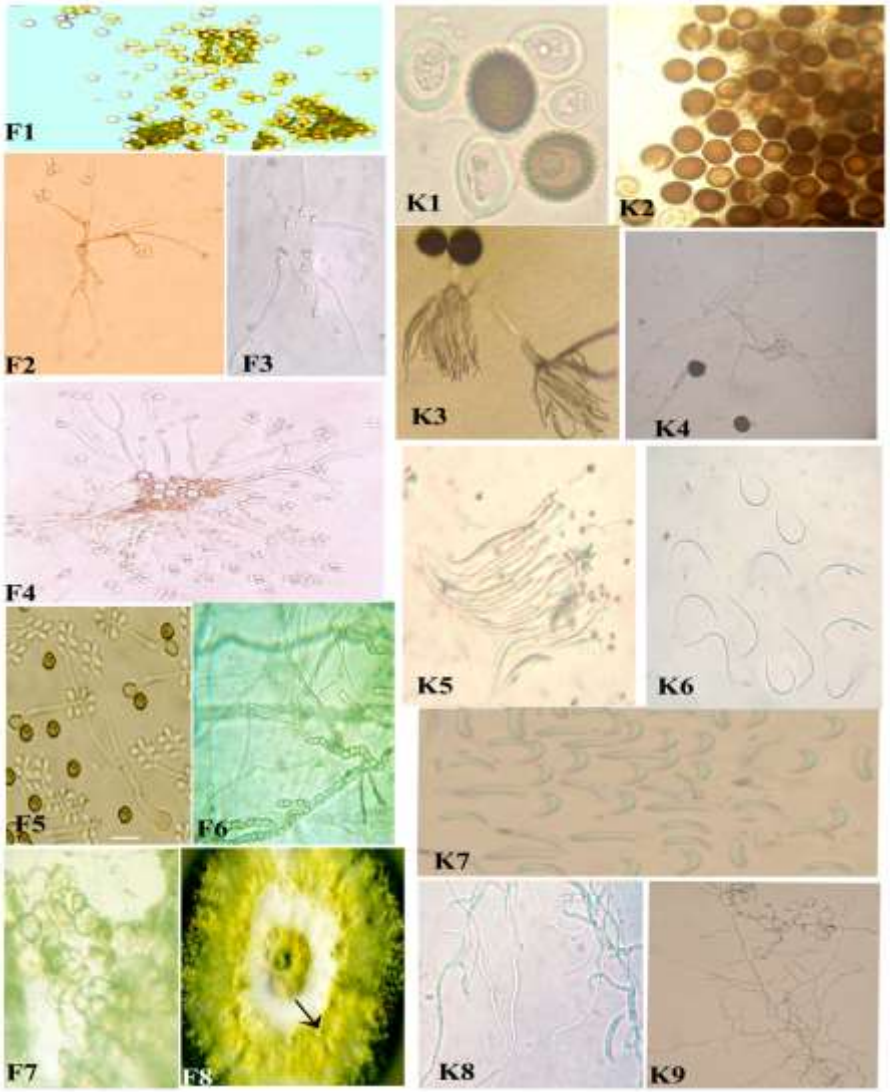


**Fig. 1 images of the rice false and kernel smut fungi. False smut (F) F1, chlamydospores, F2, germination of single chlamydospores of white isolate, F3, germ tube of secondary conidia, F4, germination of single chlamydospores of olive isolate, F5, conidiophores bearing conidia sympodially at the tapering apex, F6, false smut hyphae, F7, mycelium of false smut with thick cell wall, F8, growth of false smut on media , arrow refer to spore mass., Kernel smut (K) K1, K2, Spores and sterile cells of *T. barchlayana* on rice; K3, chlamydospores germination and promycelium bear basidiospores; k4; K5, basidiospores; K6, filliform sporidia; K7 , allantoide sporidia; K8, allantoid sporidia germination and produce the invasive mycillium of rice embryo, K9, single filliform sporidia germination.**

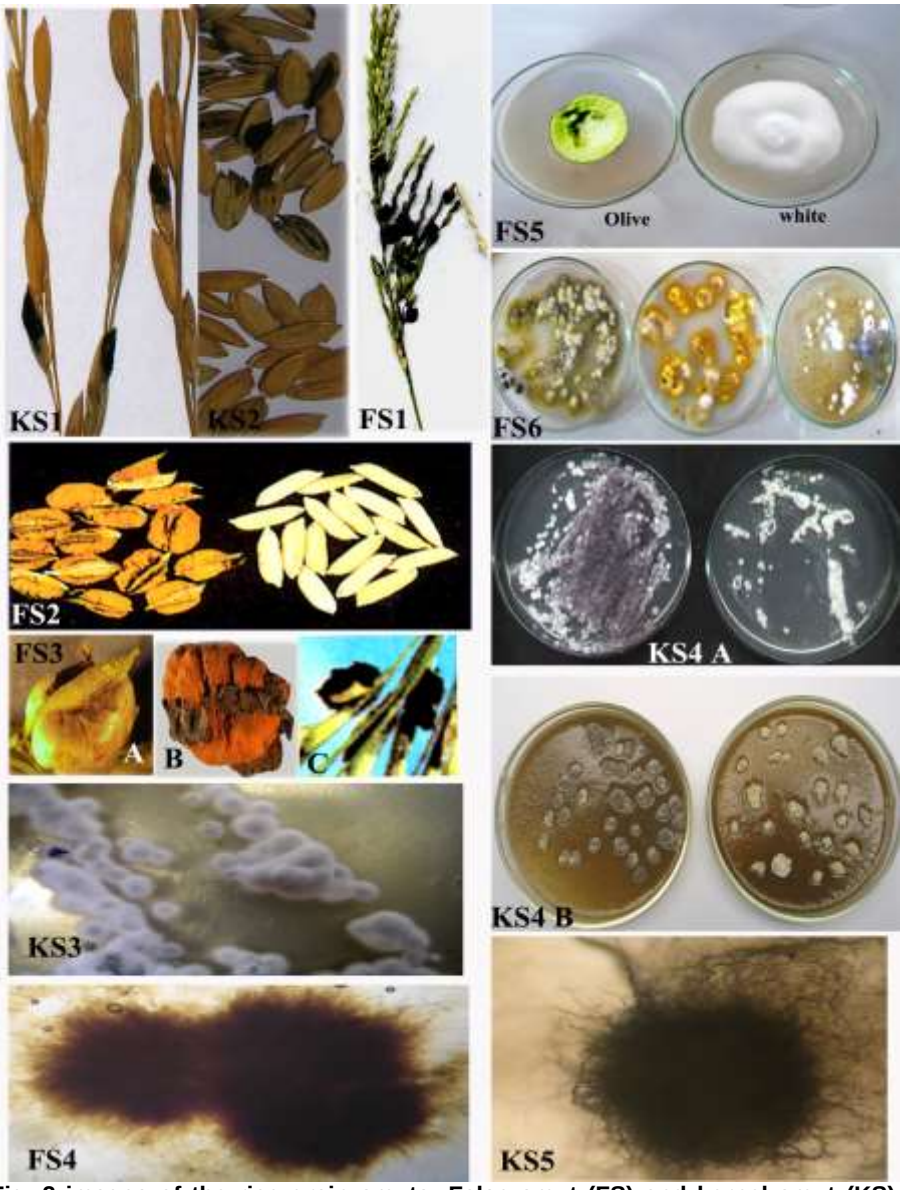


**Fig. 2 images of the rice grain smuts; False smut (FS) and kernel smut (KS), KS1, symptoms of panicles, KS2 infected and healthy grains, false smut (FS) FS1 symptoms of false smuts, FS2 infected and healthy grains, FS3, different stage of infection of false smutted grains, A, infected grains with membrane, B grains have orange color, C, grains with mature chlamydospores and dark olive color. KS3, white colony of *Tilletia*, FS4, Individual colony under microscopy 10x, FS5 , olive and white isolates, FS6 olive, orange and white color, KS4, A, kernel smut isolates with white color and purple color- convex , KS4 B, Isolate with Paige or creamy color and embedded in media, KS5 individual colony of kernel smut under 10x.**

**Table 1: Reaction of the studied twenty three Egyptian rice varieties to false smut disease during 2009 and 2010 seasons**.

| No. | Variety | Type | Duration (day) | Reaction | No. of smutted grains/ panicle | Range of smutted galls /panicle | | Resistance % compared with Giza 171 |
|---|---|---|---|---|---|---|---|---|
| | | | | | | 2009 | 2010 | |
| 1 | Reiho | J | 144.0 | S | 4.40 | 0-4 | 0-5 | 70.80 |
| 2 | Giza 159 | J | 146.3 | S | 4.27 | 0-2 | 0-4 | 71.67 |
| 3 | Giza 171 | J | 159.0 | HS | 15.07 | 0-16 | 0-18 | 0.00 |
| 4 | Giza 172 | J | 150.0 | HS | 12.00 | 0-15 | 0-11 | 20.37 |
| 5 | Giza 175 | I/J | 131.3 | S | 4.73 | 0-3 | 0-5 | 68.61 |
| 6 | Giza 176 | J | 149.0 | S | 1.40 | 0-1 | 0-2 | 90.71 |
| 7 | Giza 177 | J | 124.7 | HS | 6.67 | 0-10 | 0-8 | 55.74 |
| 8 | Giza 178 | I/J | 135.0 | S | 6.93 | 0-6 | 0-9 | 54.01 |
| 9 | Giza 181 | I | 150.0 | S | 4.8 | 0-5 | 0-6 | 68.15 |
| 10 | Giza 182 | I | 127.0 | S | 2.60 | 0-2 | 0-4 | 82.75 |
| 11 | Sakha 101 | J | 141.0 | S | 1.07 | 0-1 | 0-1 | 92.90 |
| 12 | Sakha 102 | J | 125.0 | HS | 4.53 | 0-8 | 0-5 | 69.94 |
| 13 | Sakha 103 | J | 122.0 | S | 4.07 | 0-2 | 0-4 | 72.99 |
| 14 | Sakha 104 | J | 135.0 | S | 1.07 | 0-1 | 0-1 | 92.90 |
| 15 | E. yasmine | I | 150.0 | S | 2.53 | 0-4 | 0-3 | 83.21 |
| 16 | GZ6296 | I/J | 126.0 | S | 1.80 | 0-2 | 0-2 | 88.06 |
| 17 | GZ6522 | J | 125.0 | S | 1.27 | 0-1 | 0-1 | 91.57 |
| 18 | GZ6903 | J | 134.3 | S | 2.00 | 0-3 | 0-2 | 86.73 |
| 19 | GZ7576 | J | 127.0 | S | 1.33 | 0-1 | 0-1 | 91.17 |
| 20 | GZ7769 | J | 123.0 | S | 1.20 | 0-1 | 0-1 | 92.04 |
| 21 | GZ7955 | J | 128.0 | S | 4.87 | 0-6 | 0-5 | 67.68 |
| 22 | Hybrid1(H1) | I | 135.0 | S | 2.73 | 0-5 | 0-3 | 81.88 |
| 23 | H2 | I | 135.0 | S | 5.00 | 0-7 | 0-5 | 66.82 |
| | LSD 5% | | | | 0.828 | | | |

**R, Resistant; S, susceptible; HS, highly susceptible; MR, Moderately resistant.**
**I, indica type; J, Japonic; IJ, indica japonica, E., Egyptain**

Data presented in Table (1) show that all tested varieties were susceptible to false smut and exhibited different levels of resistance. Giza 171, Giza 172, Giza 177 and Giza 178 were the highly susceptible varieties viz: although Giza 171 and Giza 172 (20.37%) are late mature cultivars 159 days. Giza 177 showed 55.74 % resistance as it is an early maturing variety 125 days. These results were in accordance with the data obtained by (RRTC, 2008). The results also show a wide range of variability in the response of different rice types against false smut infection. The resistance level of japonica type was ranged from 20.37 to 92.90 %. While, the resistance level of indica rices was ranged from 68.15 to 83.21 %. The reaction of hybrid rice was similar to indica-type behavior whereas the resistance level ranged from 66.82 to 81.88 %. Indica- japonica resistance was ranged from 54.01 to 88.06 %. The resistance of GZ lines ranged from 67.68 to 92.04 %. of the widely cultivated varieties, Sakha 101 appeared to be more resistant than the others and also exhibited high degrees of field resistance to false smut to recorded 92.90 % compared with the highly susceptible Giza 171. Also, Sakha 104 as a second extensively grown varieties exhibited a low level of false smut infection and the same resistance level. Those results are in agreement with (Ou, 1985; Singh *et al.* 1987; Deng 1989; Singh and Khan 1989; Yaegashi *et al*. 1989; Rush *et al*. 2000 and

Biswas 2001) they reported that rice cultivars played an important role in the degree of false smut infection. Furthermore, early maturing rice genotypes escaped from infection, while the late maturing genotypes did not. Also, there are varietal differences in false smut incidence and severity of disease on cultivars planted in the same sites. However, these differences appear to be from different maturity dates instead of true varietal resistance. The control of false smut disease can be depends on hygiene and use of disease-resistant cultivars. Finally, based on the response of the tested Egyptian varieties to false smut, the rice breeders can depend on varieties with high level of resistance in their breeding program for false smut disease.

**Table 2: Reaction of the studied twenty three Egyptian rice varieties to kernel smut disease during 2009 and 2010 seasons**.

| No | Variety | Type | Duration (day) | Reaction | No. of smutted grains/panicle under artificial inoculation | Range of smutted grains/panicle under natural infection | | Resistance % compared with Giza 171 |
|---|---|---|---|---|---|---|---|---|
| | | | | | | 2009 | 2010 | |
| 1 | Reiho | J | 144.0 | S | 4.67 | 0-2 | 0-1 | 74.06 |
| 2 | Giza 159 | J | 146.3 | HS | 11.67 | 0-7 | 0-6 | 35.17 |
| 3 | Giza 171 | J | 159.0 | HS | 18.00 | 0-12 | 0-10 | 0.00 |
| 4 | Giza 172 | J | 150.0 | HS | 14.67 | 0-6 | 0-8 | 22.70 |
| 5 | Giza 175 | I/J | 131.3 | HS | 10.33 | 0-5 | 0-4 | 42.61 |
| 6 | Giza 176 | J | 149.0 | MR | 1.33 | 0-1 | 0-1 | 92.61 |
| 7 | Giza 177 | J | 124.7 | HS | 9.00 | 0-8 | 0-6 | 50.00 |
| 8 | Giza 178 | I/J | 135.0 | HS | 11.33 | 0-7 | 0-8 | 37.06 |
| 9 | Giza 181 | I | 150.0 | MR | 1.67 | 0-1 | 0-2 | 90.72 |
| 10 | Giza 182 | I | 127.0 | MR | 1.67 | 0-1 | 0-2 | 90.72 |
| 11 | Sakha 101 | J | 141.0 | MR | 1.00 | 0-1 | 0-1 | 94.44 |
| 12 | Sakha 102 | J | 125.0 | S | 6.33 | 0-4 | 0-4 | 64.83 |
| 13 | Sakha 103 | J | 122.0 | S | 5.33 | 0-3 | 0-4 | 70.39 |
| 14 | Sakha 104 | J | 135.0 | MR | 1.33 | 0-1 | 0-1 | 92.61 |
| 15 | E. yasmine | I | 150.0 | MR | 1.00 | 0-1 | 0-1 | 94.44 |
| 16 | GZ6296 | I/J | 126.0 | MR | 1.00 | 0-1 | 0-1 | 94.44 |
| 17 | GZ6522 | J | 125.0 | MR | 1.33 | 0-1 | 0-1 | 92.61 |
| 18 | GZ6903 | J | 134.3 | MR | 1.33 | 0-1 | 0-1 | 92.61 |
| 19 | GZ7576 | J | 127.0 | MR | 1.00 | 0-1 | 0-1 | 94.44 |
| 20 | GZ7769 | J | 123.0 | MR | 1.00 | 0-1 | 0-1 | 94.44 |
| 21 | GZ7955 | J | 128.0 | MR | 1.00 | 0-1 | 0-1 | 94.44 |
| 22 | Hybrid1(H1) | I | 135.0 | S | 3.67 | 0-2 | 0-3 | 80.00 |
| 23 | H2 | I | 135.0 | S | 4.67 | 0-4 | 0-6 | 74.06 |
| | LSD 5% | | | | 1.111 | | | |

**R: Resistant; S: susceptible; HS: highly susceptible; MR: Moderately resistant.**
**I: indica type; J: Japonic; IJ: indica japonica, E: Egyptain**

**Varietal resistance to kernel smut disease:**

All local rice cultivars in addition to the two Egyptian hybrid rice H1 and H2 beside GZ lines were evaluated against kernel smut resistance. Data in Table 2 show that there are highly significant differences among rice varieties in their resistance to kernel smut. Old japonica short grains varieties namely; Giza 171 and Giza 172 were highly susceptible to kernel smut as Giza 171 recorded the highest no. of smutted grains/ panicle 18 followed by Giza 172, Giza 159 and Giza

178 which exhibited (14.67, 11.67 and 11.33 infected grains/ panicle) with 22.70, 35.17 and 37.06 resistance percentage, respectively. Of the widely grown cultivars, Sakha 101 appears to be more resistant than the others to exhibits high degree of field resistance to kernel smut, as it recorded 1.0 infected grain/ panicle with resistance percentage of 94.44% compared with the highly susceptible cultivar Giza 171. Also, Sakha 104 as a second extensively grown variety exhibited low level of kernel smut infection (1.33 infected grain/ panicle with 92.61%).

Indica rice varieties which distributed in restricted areas viz: Giza 182 and Egyptian Yasmine recorded the lowest infected grains/ panicle with resistance ranged from 90.72 and 94.44 %, respectively. The new release rice varieties GZ 6522 and GZ 7955 exhibited lower level of infected grains/ panicle with percentages of resistance 92.61 and 94.44 %, respectively.

Concerning the hybrid rice varieties, H1 exhibited 80 % resistance compared with the highly susceptible cultivar Giza 171, while H2 was more susceptible than H1as it recorded 74.06 %.

Giza 171, Giza 159 and Reiho as a japonica short grain which the oldest rice cultivars in Egypt were previously distributed on large scale allover rice governorates so the pathogen produced different compatible races to both. As a result of large interaction with the host they became highly susceptible. Giza 171,Giza 159 and Reiho were late maturing cultivars (late heading date), so the high inoculum density (initial inoculum sources) was provided from early maturing cultivars.

Giza 178 was a highly susceptible cultivar specially in Belkas location whereas it was grown on large areas for many years and the fungus may produce highly host-specific races. The obtained results are in agreement with those results of Templeton (1967) who indicated that some cultivars are susceptible to *Tilletia* barclayana, while zenith, Tauching netve 1 and Vista were resistant cultivars to the pathogen, Hassan (1971) tested 364 rice cultivars against kernel smut and found that only 7of them were resistant as they showed (0.2% grain infection) and while 29 cultivars were moderately resistance (0.3-1.5%). Rice cultivars ‘Cypress’, LaGrue, M204, ‘Alan’, ‘Jackson’, and ‘Newbonnet’ have been previously identified as either highly susceptible or susceptible to kernel smut (Cartwright *et al*., 1997).

For long grains including Giza 182 and Egyptian yasmine were restricted in small scattered areas these might have not opportunity for the pathogen to interact fairly with the host. As a result of this weak interaction both cultivars could be the reason of exhibiting high resistance percentage.

Although, Sakha 101 and Sakha 104 the japonica short grains were covered almost in 70 % of total rice cultivated fields, they recorded the lowest infected grians /pancile. The obtained results are in contrary with Biswas, 2003 who found that the long-grain rice cultivars which are predominant type grown in the United States were the most susceptible ones to T. horrida, compared to short grain rice which are the least susceptible cultivars. Moreover, Tempelton (1960) reported that with

some exceptions, the long-grain varieties were more severely affected by kernel smut than the short or medium-grain varieties. Also, rice which headed late in the growing season had a higher incidence of kernel smut than the earlier heading ones (Tempelton, 1967).

In spite of Giza 177, the large scale cultivated cultivar in Egypt it exhibited a highly susceptible reaction although it was an earliest maturing cultivar. The obtained results are in agreement with Chauhan and Verma (1964) who noted that early maturing cultivars were more susceptible than later maturing ones in India. All cultivars inoculated at flowering stage were in agreement with the funding of (Cartwright *et al*., 1994) who reported that kernel smut infects the open flower at anthesis and then grows in the developing kernel. In conclusion, there are different resistance levels among Egyptian rice varieties and this results are in agreement with the authors (Tempelton 1960; Durán and Fischer, 1961; Singh and Pavgi, 1970; Blaskova, 1996 and Biswas, 2001). Baloch and Bhatti, (1977) tested seventeen promising lines against kernel smut under field conditions and found that disease incidence was varied from variety to another and from year to year and the maturity period had no marked effect on the disease incidence. Chauhan and verma , 1964 and Mathusamy and Ahmed (1977) reported that early maturing varieties were more susceptible than medium and late maturing ones in india and that perhaps because of favorable conditions for infection at the time of flower opening.

From previous results it is concluded that, the successful crosses for management of rice kernel smut may depend on all highly resistant levels viz: Sakha 101, Sakha 102, Sakha 103, Sakha 104, GZ 6522, Giza 182 and Egyptian yasmine. The latest heading cultivars had higher incidence of kernel smut than the earliest heading ones as shown also by (Tempelton *et al*. 1967).

**Table 3. Growth characters of rice kernel and false smut isolate on different media.**

| No. | Media | False smut | | | | Kernel smut | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Linear growth cm (14days) | Fresh weight (g) | Dry weight (g) | No. of spores/ml | Linear growth cm (14 days) | Fresh weight (g) | Dry weight (g) | No. of sporidia /ml | No. of sporidia /ml |
| 1 | PDA | 1.3 | 6.6 | 1.5 | 39.5 | 2.9 | 7.3 | 1.8 | 48.3* | 30.6* |
| 2 | PSA | 1.5 | 8.4 | 2.4 | 66.3 | 5.5 | 14.5 | 3.3 | 94.3 | 88.3 |
| 3 | RBA | 3.1 | 15.4 | 4.9 | 71.3 | 3.6 | 10.4 | 2.4 | 66.6 | 60.2 |
| 4 | BDA | 1.8 | 6.6 | 1.7 | 37.3 | 3.4 | 8.3 | 1.9 | 60.4 | 45.3 |
| 5 | OMA | 2.1 | 12.5 | 3.9 | 60.5 | 4.5 | 11.6 | 2.5 | 71.6 | 64.7 |
| 6 | RFA | 2.3 | 11.9 | 3.6 | 57.5 | 4.2 | 12.3 | 2.7 | 82.4 | 74.8 |
| | L.S.D. 5% | 0.026 | 0.827 | 0.752 | 8.136 | 0.838 | 1.696 | 0.508 | 7.943 | 6.424 |

***no of spores/ml × $10^{-4}$**

The growth behavior of false smut fungus were significantly varied on the tested media as it was grown abundantly on rice bran medium followed by rice flour agar medium and oatmeal. The linear growth was 3.1, 2.3 and 2.1cm, respectively. For the sporulation capacity, the highest spore number was obtained from rice bran (RBA), followed by potato sucrose agar (PSA) and oatmeal agar (OMA). For fresh and dry weight, the same media was suitable for harvesting the highest fresh and dry weight, Table 3. These results in accordance with those of Tahoon (2005) who reported that the maximum growth of the fungus was occurred on rice bran dextrose agar medium, which show a higher significant difference over the other media.

Concerning kernel smut, potato sucrose agar showed to be the most suitable media for the measurements of linear growth, fresh and dry weight and the sporulation, followed by rice flour and oatmeal agar, (Table 3). However, Chahal *et al*., 1999, reported that potato dextrose agar and host-extract media supported maximum radial growth of tilletia barclayana, followed in sequence by Richard's, Czapeck's and glucose nitrate media. Production of both fusiform and allantoid sporidia were more abundantly grown on PDA and host extract media.

**Culture color and growth nature of kernel smut isolates:**

Based on culture color, growth nature and production of pigments on medium, the 10 isolates of *Tilletia barclayana* were characterized and formed three groups i.e., A, the colony has white cottony color and powdery, while the colony was convex and raised over the surface of media. The group B, was white- smooth leathery surface and raised, deeply imbedded and incorporated within the surface media similar to yeast in its growth nature. C, characters were as in group B but the colony has creamy color, (Table 4). Group A which included 6 isolates were the common observed group representing 60 % of the total tested isolates. While, group B included one isolates out of ten to represent only 10%. The group C included 3 isolates presented 30 % of ten tested isolates.

For the production of pigments, the isolates of the kernel smut were classified into three categories; the first one produced violet (bluish purple) pigment, the second gave a reddish pigment and the last one did not produce any pigments. The isolates of *Tilletia barclayana* differed in all growth characters. The isolate no. 3 from hybrid 1 recorded the highest linear growth, while, isolate no. 6 gave the highest fresh and dry weight. For sporulation capacity, the isolate no. 8 gave the highest no. of filiform sporidia / ml, while isolate no. 9 produced the highest no. of allantoid sporidia/ ml. as a result of variability in all growth characters. All isolates differed in no. of smutted grains/ panicle as a source of isolation, (Table 4). So far, it is concluded that rice kernel smut fungus has a remarkable variability level when grown in vitro. Levy *et al.*(2001). reported that Sequences of *T. barclayana*, were highly diverged. Within *T. barclayana*, isolate no. 828 differed from the isolates no. 832 and 637 by five substitutions, isolate no. 832 differed from 828 and 637 by two substitutions, and isolate no. 637 differed from 828 and 832 by two substitutions.

4-5

The culture color, growth characters and the sporulation capacity of the 10 isolates of *Ustilaginoidea virens* were characterized by the auther to include three main groups i.e., A, olive; the group B was white, while, C gruop, has orange color. The group A included 6 isolates and was the most common group representing 60 % of the total tested isolates. While, group B presented 20% of the total tested isolates. The white isolates were isolated from pseudosclerotia of the white gray color membrane. The white isolates recorded the highest linear growth on the media and these isolates were faster in their growth than the olive isolate or the orange ones. Also, white isolates recorded the highest fresh and dry weight. On the other hand, these isolates produced the lowest no. of conidiospores /ml, (Table 5). As a result of the different levels of variation among all isolates of false smut, the no. of smutted grains /panicle was differed. These results are in agreement with the funding of Tahoon (2005) who reported that the maximum linear growth was observed on the white isolates with highly significant difference to olive-green

Subsequently it could conclude that *Ustilaginoidea virens* fungus exhibited a noticeable variability range of different growth characters.

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# تقييم المقاومة الصنفية والصفات الفسيولوجية لمرضي التفحم الكاذب والتفحم الحبي للأرز في مصر

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**أمراض الأرز- مركز البحوث و التدريب في الأرز- معهد المحاصيل الحقلية - مركز البحوث الزراعية**

ينتشر مرض التفحم الكاذب في معظم أنحاء العالم وتحت كل النظم البيئية. تعتبر أمراض تفحم الحبوب من عوائق الأنتاج في الأرز وتعتبر أمراض تفحم الحبوب من الأمراض الثانوية للأرز في مصر. يصيب مرض التفحم الكاذب بعض حبوب السنبلة مما يسبب خسارة في المحصول بالإضافة إلي أنه يفرز سماً فطريا يسمي يوتلكسين والذي يؤثر علي صحة الانسان والحيوان. وأيضا يسبب مرض التفحم الحبي نقص في المحصول وجودة الحبوب. وتهدف الدراسة الحالية إلي تحديد مصادر للمقاومة لكلا من مرض التفحم الكاذب والحبي وأيضا دراسة الصفات الفسيولوجية لكلا الفطرين. تم تقييم 24 تركيب وراثي من الأصناف والسلالات المصرية لكلا المرضين تحت ظروف الحقل في مزرعة محطة البحوث بسخا في موسمي 2009 و 2010. بالنسبة لمرض التفحم الكاذب قد أظهر نتائج البحث أن معظم السلالات والأصناف المختبرة حساسة للمرض وأظهرت مستويات مختلفة من المقاومة. وكانت الأصناف جيزة 171 ، جيزه 172 ، جيزة 177 وجيزة 178 شديدة الحساسية للأصابة و بالرغم أن جيزة 171 و جيزه 172 متأخرة النضج و جيزة 177 مبكرة النضج. ويوجد مدي واسع من التنوع في أستجابة الأصناف لمرض التفحم الكاذب .وقد تراوح مستوي المقاومة للأصناف ذات الطراز الياباني من 20.37 % إلي 92.90 % بينما تراوح مستوي المقاومة للأصناف ذات الطراز الهندي من 68.15 - 83.21 %، وأيضا أظهرت أصناف الأرز الهجين نفس السلوك للأصناف الهندية الطراز وتراوحت مقاومتها من 66.22 – 81.88 %. وبالنسبة للأصناف ذات الطراز الهندي –الياباني تراوحت مقاومتها من 54.01 – 88.06 % ، وكان مستوي المقاومة للسلالات الجديدة المستنبطة من 67.68 – 92.04 % وبالنسبة للأصناف الأكثر أنتشارا وهي سخا 101 ، سخا 104 كانت أكثر تحملا للإصابة حيث أظهرت 92.90 % مقاومة مقارنة بالصنف الحساس جيزة 171.

وبالنسبة لمرض التفحم الحبي كانت الأصناف جيزة 171 ، حيزة 172 ، جيزة 159 ، و جيزة 178 أكثر الأصناف حساسية لهذا المرض بينما كانت الأصناف سخا 101 ، سخا 104 ، جي زد 6522 ، جي زد 7955 والصنف جيزة 182 والياسمين المصري متوسطة المقاومة بنسبة تزيد عن 90 % .

ومن ناحية أخري فقد أظهرت النتائج أن أنسب بيئة لنموتجرثم فطر التفحم الكاذب رجيع الكون بينما كانت بيئة مستخلص البطاطس المضاف إليها سكروز أنسب البيئات لنمو وتجرثم فطر التفحم الحبي.

أظهرت الدراسات المعملية لزراعة هذه الفطريات أن عزلات كل من الفطرين ذات مستوي عالي من التنوع حيث أختلفت عزلات فطر التفحم الكاذب من حيث اللون الزيتوني والأبيض والبرتقالي بينما أختلفت عزلات فطر التفحم الحبي فقد كان الاختلاف واضحا من حيث أنتاج الصبغات لتتباين الصبغات بين البنفسجي والحمراء الداكنة.

**قام بتحكيم هذا البحث**

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**Table 4. Growth characters of rice kernel smut isolates from different governorates and cultivars on potato sucrose agar media (PSA).**

| Isolate No. | Governorate | Location | year | Cultivar | Pigment color | Growth nature | Linear growth (cm) 14 days | Fresh weight (g) | Dry weight (g) | No. of filiform sporidia /ml | No. of allantoid sporidia /ml | **No. of smutted grains/ panicle |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Kafr Elshiekh | Qallin | 2008 | Giza182 | Violet | A | 2.8 | 7.4 | 1.8 | 10.3* | 6.9* | 5 |
| 2 | Kafr Elshiekh | Sakha | 2009 | Hybrid1 | Reddish | C | 5.7 | 13.5 | 3.9 | 20.3 | 12.4 | 6 |
| 3 | Sharkia | Zagazig | 2009 | Sakha101 | No | A | 4.5 | 14.4 | 4.1 | 67.8 | 56.8 | 1 |
| 4 | Sharkia | Abu-Kabeer | 2010 | Sakah104 | Violet | C | 3.6 | 8.3 | 1.7 | 53.2 | 44.6 | 1 |
| 5 | Gharbia | Gemmaiza | 2012 | Giza171 | Reddish | A | 5.3 | 11.4 | 2.8 | 45.5 | 25.3 | 7 |
| 6 | Gharbia | Qotour | 2012 | Giza177 | No | C | 3.6 | 15.3 | 4.3 | 89.4 | 69.9 | 5 |
| 7 | Behiera | Damanhour | 2008 | Giza177 | No | B | 4.8 | 12.6 | 3.5 | 6.8 | 3.5 | 1 |
| 8 | Behiera | Etai | 2009 | Hybrid1 | Violet | A | 4.3 | 10.8 | 2.5 | 147.3 | 78.4 | 2 |
| 9 | Dakahlia | Mansoura | 2009 | Giza177 | Reddish | A | 5.2 | 7.3 | 1.4 | 37.6 | 23.4 | 4 |
| 10 | Dakahlia | Belkas | 2010 | Giza178 | Reddish | A | 3.5 | 12.4 | 2.9 | 98.4 | 56.8 | 5 |
| | LSD 5% | | | | | | 0.352 | 0.292 | 0.460 | 4.409 | 2.612 | |
| | A, White-cottony powdery – Raised and Convex-like fungal growth | | | | B, White –smooth leathery-raised ,Embedded in media-like yeast growth | | | C, Creamy –smooth leathery-Raised and Embedded in media – like yeast growth | | | | |

****panicle as a source of isolate *number of spores × $10^{-4}$**

**Table 5. Growth characters of rice false smut isolates from different governorates and cultivars on rice bran agar media (RBA).**

| Isolate No. | Governorate | Location | Year | Cultivar | Culture color | Linear growth (14 days) | Fresh weight g | Dry weight g | No. of Conidio-spore/ml | No. of smutted grains/panicle |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Kafr Elshiekh | Qallin | 2008 | Hybrid1 | Olive canary | 3.1 | 16.4 | 5.4 | 143.5 | 3 |
| 2 | Kafr Elshiekh | Sakha | 2009 | Giza182 | Dark Orange | 2.4 | 7.4 | 1.5 | 48.6 | 2 |
| 3 | Kafr Elshiekh | Sakha | 2010 | Giza178 | Olive-green | 1.8 | 9.4 | 2.4 | 124.5 | 4 |
| 4 | Sharkia | Zagazig | 2009 | Sakha101 | White | 3.8 | 6.2 | 1.4 | 25.4 | 1 |
| 5 | Sharkia | Abu-Kabeer | 2010 | Sakah104 | Olive | 2.7 | 11.5 | 3.2 | 96.8 | 1 |
| 6 | Sharkia | El-hosainia | 2010 | Giza178 | Olive | 2.3 | 6.4 | 1.5 | 115.4 | 9 |
| 7 | Gharbia | Gemmaiza | 2012 | Giza171 | White | 4.5 | 13.6 | 3.5 | 35.4 | 17 |
| 8 | Gharbia | Qotour | 2012 | Giza177 | Dark olive | 2.5 | 8.6 | 1.8 | 73.7 | 10 |
| 9 | Gharbia | Basion | 2012 | Sakha 103 | Olive | 1.9 | 12.3 | 3.4 | 123.3 | 7 |
| 10 | Gharbia | El-Santa | 2012 | Sakha102 | Light orange | 1.5 | 15.4 | 5.2 | 86.7 | 8 |
| 11 | LSD 5 % | | | | | 0.636 | 2.381 | 0.471 | 3.279 | |