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Inheritance of resistance to loose smut ustilago *tritici* of wheat

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ABSTRACT

Ustilago tritici, the causal agent of loose smut of wheat (*Triticum aestivum L*), belongs to family Ustilaginaceae. In nature, infection by *Ustilago segetum* var. *tritici* is organ specific i.e. via the ovary, and it is possible only during the relatively short period of flowering of the host. In the field, there are two methods of inoculation; dry method and wetable method. An extensive work has been done in many countries of the world for identifying sources of resistance to that disease, since breeding for resistance may be one of the most effective methods for controlling the disease. Loose smut causes a reduction in agronomic charactesrs. Yield is an ultimate product of the action and interaction of a number of quantitative characters, which are known to be controlled by different sets of polygenes. Therefore, ombining ability studies are used by plant breeders to select parents with maximum potential of transmitting desirable genes to the progenies. The estimation of combining ability is very useful because the variance due to general combining ability is attributed to additive gene action, while the variance due to specific combining ability is attributed to non-additive gene action. The inheritance of heterosis could be help to select some superior parents and cross combinations for further exploitation in breeding programme either agronomic traits or resistance to plant disease and proved that resistance was dominant to susceptibility. The results indicated at least one major gene and one minor gene segregation for resistance and transgressive segregation for these parents differed in genes for resistance. Improvement of quality in wheat (*Triticum aestivum* L.) depends on the influence of environmental conditions and their interactions. Loose smut causes damage by destroying the infected plants and reducing the quality of grain of the non-infected plants upon harvest. Embryo test for detecting the dormant mycelia in the infected seeds has extensive importance, since a direct correlation was observed between the level of embryo infection and the incidence of loose smut in the field. It was stated that the total nitrogen content of the host- pathogen complex generally increased during the early stage of disease. Increase in secreted protein play important role during pathogenesis and thought to act as effectors for modulating the plant response. Fungus penetration and growth through cells as well as disintegration of plant cell wall at later stages requires lytic enzymes.

INTRODUCTION

Ustilago tritici, the causal agent of loose smut of wheat (*Triticum aestivum L*), belongs to family Ustilaginaceae; order Ustilaginales; class, Teliomycetes; division, Eumycota. Loose smut is considered a serious seed-borne disease of wheat and it found in all wheat-growing areas of the world, being particularly in humid areas[1-2].

Symptoms of wheat loose smut disease

Ustilago segetum tritici is a basidiomycete's fungus that infects wheat during flowering (floral infecting smut).Teliospores are carried into the flower

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by wind and settle mainly at the shoulder of the ovary. The spores germinate and dicaryotic infectious hyphae invade the developing seed by penetration of the pericarp. The fungus grows through the seed coat into the scutellum and embryo at the base of the seed[3-5]. After germination of the seed, the mycelia permeate the crown node and enter the growing point of the tillers. The fungus is carried passively up with the plant growing point, which eventually develops into a smutted ear[3]. Neither the infected seed nor the developing plants show any obvious or unambiguous macroscopic symptoms until appearance of infected ears[6-8].

Epidemiology of loose smut in Egypt

Wheat has been attacked with many destructive diseases such as rusts, smuts, mildews and some other diseases of minor importance[9-12]. In Egypt, wheat loose smut, caused by *Ustilago segetum* var. *tritici* ranked the second serious disease following rusts. The first record of wheat loose smut caused by *Ustilago tritici* was in 1888 as a result to exchange of seed between countries. In 1949, the losses of wheat yield ranged between trace to 5% on the local and Indian varieties. Since that date, the disease disappeared for long time due to realizing the resistant varieties Giza -139, Giza- 144 and Giza 155[13-16]. Then, it was recorded at 1984/1985 on some varieties Sakha 61, Sakha 69 and Giza-163. During the period from 1985-1989, annual loose smut survey was performed in 16 Governorates, revealed that disease incidence was about 0.1% on the commercial varieties i.e. Sakha 61 and Sakha -69[17-20].

Methods of inoculation with loose smutIn nature, infection by *Ustilago segetum* var. *tritici* is organ specific i.e. via the ovary, and it is possible only during the relatively short period of flowering of the host[21-24]. Teliospores enter the floret, germinate and form dikariotic hyphae that infect the ovary, usually at the brush end[9,25,28]. The mycelium enters the upper and side parts of the scutellum 10 to 15 days after penetration and grows through the hypocotyls into the plumular bud, or growing point of the embryo, where it will lie dormant in the mature seed. Most of field inoculation methods developed to simulate natural conditions. There are two methods of inoculation.

Dry method

For inoculation, dry spores of loose smut are introduced into the florets with forceps or a small brush, by a puff of air over tiny pieces of paper or ball of cotton containing spores, or by dusting spores over entire spikes[29-32]. Other inoculation method inculde: partial vacum[33-37] air blast[38-40]. Joshi *et al*.[41] developed this method by removing of central floret of each spikelet and clip the glumes of the two remaining florets to expose the stigma and anthers then cover the spike with a paper bag and before anthesis is complete use a smutted spike to dust teliospores onto the clipped spike. Pandey and Gautam[42] stated that the dry spore method for inoculation with loose smut (*Ustilago segetum* var.*nuda)* seems adequate for initial germplasm screening and inheritance studies but for more rigorous screening of advanced breeding material the modified partial vacuum method may be preferred.

Wetable method

The spore suspension was injected into the floret using a rubber ball[43-47] or a syringe and hypodermic needle[48-50]. Zadoks *et al*.[51] stated that inoculum of individual races was prepared at a rate of about 1 mg of spores per 1 ml of tap water. At anthesis growth stage, individual florets were inoculated with the spore suspension using a 10- ml syringe to pierce the palea. Wilcoxson *et al*.[52] used vacuum procedure for inoculation of oat (*Avena sativa*) parental varieties and lines and progenies (*Ustilago avenae*) with water suspensions of loose smut teliospores. Willits and Sherwood[53] used a modification of the vacuum inoculation method in which seeds of each barley cultivar were dehulled and surface-sterilized for 5 min in 5.25% sodium hypochlorite. A total of 30 seeds were inoculated by adding approximately 25 mg of race 8 teliospores to the seeds with 0.5 mg of carboxy methylcellulose and 1 ml of sterile water containing 0.001% Tween 20. The seeds were vacuum-infiltrated three times for 10 min, dried, and planted in damp vermiculite at a depth of 3 cm. Mau *et al*.[54] inoculate plants at anthesis with loose smut when the first anthers of the mid – spike had extruded and started to turn white. Inoculum was prepared by placing a piece of an in-

fected spike into 50 ml bottle with 10 ml of distilled water.

Time of inoculation

After heading, plants with sporulation stop growing. The lower internodes are usually longer and the upper ones shorter than in healthy plants, but the peduncle of the spike with sporulation is much shorter. The leaf sheaths of some infected cultivars are greyish-purple; the leaves, particularly the flag leaf, are reduced in size, often yellowed and senesce early[55-57]. For maximum infection, the wheat floret inoculated at early mid- anthesis growth stage. At this stage, the first anthers of mid-spike had extruded and started to turn white[58]. Ohms and Bever[59] inoculated individual heads of the winter wheat var. Wabash (C.I.11384), with race 3 of *U .tritici*, starting 2 days after anthesis and ending 5 days after anthesis. They found that the highest percentage of infected embryos was recorded at anthesis, while those rates decreased from earlier and / or later inoculation. Loria *et al*.[60] also found that florets inoculated during anthesis were 3.1- 3.8 times more susceptible than those inoculated before or after anthesis. Beniwal and Karwasra[61] found that early inoculation of 3 wheat varieties with *Ustilago nuda var. triticiUstilago segetum var. nuda*] when the ears were started to emerge. Also, Jones and Dhitaphichit[62] compared the floret and seedling inoculation method of wheat and barley with *Ustilago tritici* and *Ustilago nuda.* They found that wheat gave higher infection levels with floret inoculation method than seedling inoculation method. Kaur *et al*.[63] suggested that the dry twist method was the best inoculation method. The best growth stages (GSs) for inoculation with the pathogen was GS 65 in wheat cultivars WL 711 and HD 2329, GS.60 in durum wheat cv. PDW 215 , and GS 55 in triticale variety TL 2436.

Sources of resistance to wheat loose smut

Breeding for resistance to wheat loose smut may be one of the most effective methods for controlling the disease. An extensive work has been done in many countries of the world for identifying sources of resistance to that disease[64-69]. A variety may be highly susceptible in the embryos stage to a race of loose smut and highly to the same race in seedling and adult plant stages. Ohms and Bever[70] found that Kawval wheat cv. was resistance to 3 physiologic races of *Ustilago tritici* viz. 1, 3, and 11. On the other hand, Wabash wheat cv. was susceptible to race 1 and 3 but resistance to race 11. No differences were observed between both of tested embryos of the two cvs., Kawval showed infection in scutella only while the latter exhibited infection in both scutella and growing point. Mishra *et al*.[71] stated that of 92 cultivars screened against *U. Segetum* var. *tritici* by needle inoculation during 1980-82, 15 were designated as resistant and 3 as moderately resistant. Gupta *et al*.[72] evaluated 938 *T. aestivum*, *T. durum* and triticale lines for resistance to loose smut from 1982 to1989. Twenty-two lines and 19 of which were completely free from loose smut for 3 or more years. Beniwal *et al*.[73] evaluated 2190 wheat (*Triticum spp.]* cultivars for reaction to loose smut (*Ustilago segetum* var. *tritici)* at Hisar during 1981/82 to 1996/97. Of these, 307 genotypes were free of infection during different crop seasons, but only 99 (comprising 52 *T.durum*, 44 *T.aestivum*, 2 *triticale* and one *T.dicoccum*) maintained resistance for 5 or more years. Singh *et al*.[74] screened a total of 931 advanced lines of *T.eastivum* (802), *T. durum* (95), *T.dicoccum* (8) and triticale (26) against loose smut (*U.segetum* var. *tritici*) under artificially inoculated conditions at Hisar (Haryana) and Ludhiana (Punjab), India during the 1992 / 93 -1998 / 99 cropping seasons. Ninety-nine lines (22 *T. eastivum*, 55 *T. dicoccum* and 17 triticale lines) were resistance to loose smut, showed infection ranging from 0 to 5%. Rathod *et al*.[75] screened a total of 120 wheat strains under natural conditions. The results revealed that only 17 strains showed trace infection, where, 2 strains (N-59 and Lok1) showed susceptibility to loose smut of wheat. The majority of the strains were observed to be free from the disease strains. Gothwal and Pathak[76] evaluated 168 varieties using artificial inoculation with teliospore mixture of 103 isolates of *U. tritici*. No one of them was immune, the least infection was (3.2-5%) in WG -430 JH -102, NP-818, CPAN-722 and C-217. Sharma *et al*.[77] evaluated 439 *Triticium*

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aestivum lines and 13 T.durum against a mixture of Indian field races of *Ustilago tritici*. The commonly cultivated HD-2009, WH-147, WL-711, Sonalika, Kalyonsona and WL-1562 of the bread wheat lines were susceptible. Mean while, 10 of the 13 -*T.durum* lines were resistant. Kiseleva[78] examined accessions of spring bread wheat, spring durum wheat and oats for resistance to *Ustilago tritici* in wheat and avenae (in oat) under field and green-house conditions during 1983-88. He found that inoculation in the green- house promoted better manifestation of the disease than field infection. The least disease incidence was shown by Leucurum -120 among durum wheat and Biryasink x Omshaya -3889 among bread wheat. Wherever, Dula x21h-263, Narymshil x frazer and falenski x 20/1268 were the least infected oats. Mishra *et al.*[71] screened 92 varieties against *U. segetum* by needle inoculation in 1980–82. Fifteen lines were R (resistant) and three were rated MR (Moderately resistant). Sherif *et al.*[79] tested 96 wheat entries as well as 10 Egyptian wheat varieties to loose smut and found that 14 entries were highly resistant. The Egyptian wheat cvs .Giza 155, Giza160 and 162 were resistant (0-5%), while Sakha 61 and Sakha 92 were susceptible. Giza 157 Sakha 69 cvs were moderately susceptible (11-20%) while, Giza 163, Giza 164 and Sakha 8 were moderately resistant (6-10%). Rewal[80] suggested that the suppression of tillering associated with smut infection should be used as a supplement to ear infection in estimating the disease. El–Shamy and Hamada[64] tested five new commercial Egyptian varieties namely, Sids-1, Sids-6, Sids-7, Sids-8, Sids-9 against artificial inoculation with wheat loose smut and the result compared with the cvs. Giza-155 and Sakha-61. All the tested varieties showed susceptible reaction ranged from 18.54-39.68 % and 16.66-30.25% during 1998 and 1999 growing season. Sakha- 61 showed the highest disease incidence either in embryo test or under plastic green house; where as Giza 155 cv. was free at adult plant. Thomas[81] found that an Ethiopian accession, CI 9973 was resistant to many different isolates of loose smut and therefore, could be an excellent source of loose smut resistance.

Effect of loose smut on agronomic characters

Beniwal *et al.*[82] studied the effects of *Ustilago nuda tritici* on tiller height, number of tillers, number of smutted tillers, ear length and flag leaf in 8 cultivars. They found maximum reduction in tiller height occurred in Sone and Max cultivars, reduction in length of ears in the cultivar Kalyansona. The total number of tillers was reduced in all the cultivars and the incidence of smutted tillers ranged from 17.7-61%, according to the cultivar. Lal and Siddiqui[83] found that Infection of wheat by *Ustilago nuda* f.sp. *tritici* reduced the average number of tillers produced per plant [6.3 compared with 11.3 on healthy plants]. Ahmad *et al.*[84] reported that infection by *U. Segetum* var. *tritici* resulted in the destruction of the whole panicle and also affects various growth components. In experiments with 6 genetically different cultivars, there was a 5-14% decrease in plant height, 22-53% decrease in number of tillers/plant, and 15-29% decrease in dry stem wt, but the number of leaves/plant was unaffected.

Inheritance of plant characters

Yield is a complex character and is an ultimate product of the action and interaction of a number of quantitative characters, which are known to be controlled by different sets of polygenes. The choice of parents is a very important task in a breeding program. Combining ability studies are used by plant breeders to select parents with maximum potential of transmitting desirable genes to the progenies. The estimate of general combining ability (GCA) are very useful because the variance due to general combining ability is attributed to additive gene action, while the variance due to specific combining ability is attributed to non-additive gene action[85]. Bhatt[86] studied the inheritance of heading date, plant height, and kernel weight in two crosses of spring wheat (*Triticum seativum* L.*)* each involving three cultivars. He found that the F1 means were intermediate between the two parental means, but were nearer to the low parental mean, indicating partial dominance of genes controlling earliness in heading. The heritability in the narrow sense was smaller in magnitude than the corresponding broad sense ones. Esparza-Martinez and Foster[87] reported that the re-

lationship between yield and heading date was not consistent among crosses and positive r values were quite low. Heterosis over the mid-parent was quite similar among crosses for heading date, but there was no heterosis over the high parent.

Heterosis

Heterosis is estimated as a percentage of F1 over mid and / or the best parents. The inheritance of heterosis could be help to select some superior parents and cross combinations for further exploitation in breeding programme either agronomic traits or resistance to plant disease . There is a shortage in published papers about heterosis for resistance to wheat loose smut, while about yield and other traits of wheat has been reported by Khanzada *et al*.[88] Line x tester analysis used to estimate heterosis of some quantitative traits of wheat, Hanssan and Abd El-Moniem[89] obtained heterotic effects for earliness, spike length, number of spikes/plant, number of grains/ spike, 1000 grain weight and grain yield/ plant. Nassar[90] studied heterosis in 15 genotypes of bread wheat and obtained significant heterotic effects which were 30 and 51.11% for number of grains/ spike; 15.89 and 38.53% for 1000 grain yield/ plant over the better and mid- parent, respectively. Hendawy[91] stated that 36 hybrid combinations showed highly significant estimates of useful heterosis ranged from 1.50 to 30.4% for grain yield/ plant over the respective better parent. Singh and Prasad[92] used line x tester analysis to study heterosis for quantitative traits in 10 lines and 4 testers. They found variation in heterosis for yield/ plant ranged from 8.79 to 41.14 %. Rasul *et al.*[93] reported that grain yield / plant showed the highest heterosis over the mid parent (31.56%) followed by number of grains/spike (15.56%), spike length (7.14%). Singh[94] found positive heterosis heading date, number of spikes / plant, spike length, number of grains/ main spike and 1000 grain weight except plant height and grain yield/plant.

Genes conditioning loose smut resistance

Information on the number and effectiveness of genes for resistance to *U. tritici* is important for developing resistant cultivars. Several studies have been conducted to determine the inheritance of resistance to loose smut in hexaploid (common) wheat[95-100]. Pandey and Gautam[42] evaluatd parental, F1, F2 and backcross generations from crosses between 7 varieties resistant to *U. segetum* var.*tritici* (HD2236, WL2087, WL2053, WL1804, WL1798, WL1567 and WL1541) and 2 susceptible varieties [Sonalika and WL711] for loose smut disease after inoculation at mid-anthesis with a dry spore mixture of field races. Segregation ratios indicated that resistance to *U. tritici* in each of the resistant varieties is controlled by a single dominant gene. Guleria *et al*.[101] studied the inheritance of resistance to loose smut in 4 wheat cultivars and their hybrids. The segregation patterns of the F2 and backcrossed generation suggest that smut resistance is a dominant trait in CPAN2016, CPAN2099 AND PBW65 and a recessive trait in CPAN2059. Grewal *et al*.[102] studied genetics of loose smut [*Ustilago segetum* var. *tritici*] resistance in 11 bread wheat genotypes (9 resistant and 2 susceptible). F1, F2, BC1 and BC2 generation were evaluated by artificial inoculation for 36 direct and reciprocal crosses. No evidence of cytoplasmic effects was observed from the comparison between reciprocal crosses, and resistance was dominant to susceptibility. Segregation ratio indicated that ML521, WG2455, WG2753 AND W2942 carry 1 dominant resistance gene whereas WL3914, W972, WG3069 and W3902 have 2 epistatic genes, WL3203 for loose smut resistance. Knox *et al*.[95] studied the inheritance of resistance against *Ustilago* var. *tritici* in two haploid wheat populations and two inbred random head- to-row populations. The results indicated at least one major gene and one minor gene segregation for resistance and transgressive segregation for these parents differed in genes for resistance.

Effect of loose smut infection on wheat quality

Improvement of quality in wheat (*Triticum aestivum* L.) depends on the influence of environmental conditions (temperature, relative humidity, biotic and biotic factors) and their interactions. Wheat quality can be defined in terms of physiological characteristics of the grain including intrinsic properties i.e., protein and carbohydrate content[103-106]. Much

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interest has been associated with nitrogen metabolism of infected plants particularly in relation to the differences between resistant and susceptible varieties[107]. Loose smut causes damage by destroying the infected plants and reducing the quality of grain of the non-infected plants upon harvest[108,109].

Detection of loose smut mycelia in wheat embryos

Embryo test for detecting the dormant mycelia in the infected seeds has extensive importance and this item was a subject of extensive work by many authors in different locations. Popp[110] developed a method for detecting the mycelia of *U. tritici* and *U. nuda* in embryos, seedlings and adult wheat and barley inoculated plants. The tested portions were macerated in a solution of cotton blue lactophenol the mycelia was stained blue. Popp[110] described a detailed method for extracting and staining the mycelia of *U. tritici* using trypan blue. Morton[117] described a quick method (i.e. 90 minutes) for determination of loose smut of barley embryo whole mounts. He used boiling solution of 5% sodium hydroxide and 14% commercial liquid glass plus a small quantity of detergent in extraction. Boiling lactophenol served as clearing material and un-damaged embryos of high clarity were obtained. The mycelia of loose smut fungus *U. nuda* are redish-brown and can be detected with dissecting microscope. Morton[111] and Rennie[112] extract embryos from seeds by soaking in NaOH, clearing in boiling lactophenol and stained with trypan blue. Infected embryos can be identified under a dissecting microscope because of selective uptake of the stain by fungal hyphae. Bhutta and Ahmed[113] using the embryo count technique, found that of 104 wheat seed samples, 15 contained dormant mycelia of *Ustilago tritici*.

Relationship between Embryo test and Field response

When the infected seeds were sown, a direct correlation was observed between the level of embryo infection and the incidence of loose smut in the field[114-115]. Similar result obtained by Khanzada *et al.*[116] which found that any part of the embryo showing the mycelium was counted as infected, and the scutellar infection in wheat embryos was related to the number of smutted plants in the field. It is reveald a close relation between embryo test and disease expression in the field. The level of plumule but infection in the embryos of wheat is directly correlated with number of smutted plants in the field, but some infected plants also produced a few healthy tillers. Rewal and Jhooty[117] found a direct correlation between infected barley embryos and seedlings having 50% of tissue invaded by mycelia of *Ustilago nuda* and field expression of the disease. Seedling with less 50 % infection becomes free from loose smut mycelia. Ram *et al.*[118] tested seeds of 26 wheat genotypes for infection of the embryo following inoculation of the anthers with *Ustilago nuda var. tritici [Ustilago segetum var. tritici]*. Of the 26 genotypes, 5 varieties had less than 4% embryo infection, 8 were free from fungal infection, while 6 genotypes showed a high level of infection (60-84%). Jhooty and Rewal[119] revealed that, the percentage of infection with loose sumt of the wheat cultivar Sonora 64 in the growing point were reduced with the lapse of time i.e. the percentage of infection in the embryos (0 time) was 75.1% whereas it reached 62%3 weeks after emergence. They used this phenomenon to determine effectiveness of different systemic fungicides specified for controlling loose smut of wheat. Khanzada *et al.* [88] demonstrated that infection of plumular bud tissues of embryo is directly correlated to the development of smut in adult plants, the ratio being 1:1. Also, Abd El-Kader[120] found that the reaction of wheat varieties (Sakha 8, Sakha 61, Sakha 92, Gemmeiza 1, Gemmeiza 13, Gemmeiza 15, Giza 155, Giza 160, Giza 162, Giza 163, Giza 164, Giza 165, Giza 167, Giza 168) to loose smut in the field approximately runs in parallel line with reaction of embryo test. EL-Said[121] found that infection of embryos of the wheat cultivars Sakha-61, Gemmeiza-10 and Giza-155 was nearly from their field reaction to loose smut disease.

Effect of loose smut on grain protein

Grain protein content is considered a very important trait in bread wheat and has been extensively studied[122]. Grain protein content is largely affected

by environmental conditions such as soil fertility, rainfall and temperature. Generally there is an extensive work has been carried out on the biochemistry of the host – parasite interaction plant diseases, but there is a lack published information regarding the relation between wheat grain protein and the infection with loose smut. Uritani[107] stated that in fungus- infected plants, the total nitrogen content of the host- pathogen complex generally increased during the early stage of disease. Increase in secreted protein play important role during pathogenesis and thought to act as effectors for modulating the plant response[123]. Fungus penetration and growth through cells as well as disintegration of plant cell wall at later stages requires lytic enzymes[124-125]. Farahat[126] found that pea leaves and stems infected with powdery mildew have increased content of amino acids and the increase was more pronounced in the highly susceptible variety. On the contrary Omar[127] found that there was no clear relationship between the amino acid contents and changes occurring in the susceptible and resistant wheat varieties to powdery mildew disease caused by *E. graminis tritici*. Brien *et al*.[128] found that stripe rust caused wheat grains to be shrunk. The infection result in reduction in test weight and flour milling yield and increased grain protein content. Farag[129] found that amylase; lipase and protease activities were higher in the infected wheat, sesame and soybean seeds comparing with healthy seeds. Drijepondt *et al*.[130] indicated that leaf rust infection of Thatcher cv. reduced the total grain yield per plot by 25.40 % and 100-grain weight by 5.6%. Evaluation of milling and backing quality characteristics revealed that compared to Thatcher, RL - 6058 had higher flour protein content but inferior milling, dough development and backing properties. Mostafa[131] reported that the infestation of stored grains of durum and soft wheat with *A. flavus, A. niger, Alternaria alternata* and *Fusarium moliforme* reduced total soluble sugar and total crude protein content and increased fat acidity values, especially in grain with a high moisture content (17%). Wheat grains artificially inoculated with loose smut fungus showed increment in the average of protein content compared with non inoculated grains[72]. El-Shamy and Hamada[64] tested five new commercial Egyptian varieties to artificial inoculation with loose smut and the result compared with the Giza 155 and Sakha 61. The inoculated grains of the tested varieties showed insignificant increment in the average of protein content (14.27 and 14.34%) compared with the un inoculated grains (13.58 and 13.79%).

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