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## RESISTANCE OF GUAR *Cyamopsis tetragonoloba* (L.) Taub. TO HARMFUL ORGANISMS

(review)

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### Abstract

Guar (clusterbean) *Cyamopsis tetragonoloba* (L.) Taub., a tropical annual legume crop of a multipurpose use, is promising for growing in the South Russia. The problem of resistance of guar to diseases and pests is discussed. The *Alternaria* leaf blight caused by *Alternaria cyamopsidis* Rangaswami & Rao and bacteria leaf blight caused by *Xanthomonas axonopodis* pv. *cyamopsidis* (Patel) Vauterin are the most harmful diseases of guar. Seed infection promotes the extensive and fast spread of the disease. Anatomical and morphological characters are not having any relationship with *A. cyamopsidis* resistance in clusterbean plants. Sunshine, minimum temperature, cumulative rainfall and relative humidity in the evening were found significantly associated with *Alternaria* leaf blight severity (M.S. Saharan et al., 2004). The resistance of guar to bacteria leaf blight is oligogenically controlled (P.S.K. Anil et al., 2012). For *X. axonopodis* pv. *cyamopsidis* a differential interaction with plant host genotypes is characteristic. In the USA the two races of the pathogen (0 and 1) have been identified which differed not only by the virulence to guar varieties but serologically as well. A protocol of ELISA test for detecting virulent and avirulent strains of the bacteria is elaborated (G.K. Vijayanand et al., 1999). The pathogen isolates significantly differ in aggressivity when they are proliferated on resistant (HG 75) and sensitive (PNB) genotypes of guar. The analysis of the isolates with the use of molecular markers has revealed a significant polymorphism of the pathogen populations. The results obtained using two different approaches correspond to each other (B. Kaur et al., 2005). Plant infection with bacteria leaf blight and *Alternaria* leaf blight induces protective response (i.e. lignin and phenol compounds accumulation, increase of peroxidase activity). The induced resistance was observed when guar was inoculated with casual agents of charcoal rot *Macrophomina phaseolina* (Tassi) Goid., root rot *Rhizoctonia solani* J.G. Kühn, wilt *Fusarium solani* (Mart.) Sacc., and also with aphids infestation. The diversity of cultivated guar varieties for resistance to pathogens is not high. At the same time a differential interaction with plant host genotypes is revealed not only for bacteria leaf blight causal agent but also for *M. phaseolina* (S. Purkayastha et al., 2006). This means that varieties with different resistance genes should be grown for prevention of epiphytotic. The introgression of resistance genes from the wild species *C. senegalensis* Guill. & Perr. and *C. serrata* Schinz is considered as a promising approach for broadening genetic diversity (S. Kumar et al., 2017). However, interspecific crosses and phenotypic selection are the main breeding methods applied to date. In recent times the intercropping of guar with other crops (millet, okra, and castor) is also used for controlling populations of harmful organisms.

Keywords: guar, *Cyamopsis tetragonoloba*, bacterial blight, *Alternaria* leaf blight, root rot, insect pests, resistance

Guar, or clusterbean *Cyamopsis tetragonoloba* (L.) Taub. (family *Fabaceae* L.,  $2n = 14$ ), a new for Russia crop, presents great interest for selection and genetic research. The plant originates from India where its basic planted areas are concentrated; recently, it has also been cultivated in other countries of Asia, Africa, America (mainly in the USA) and in Australia. Guar is used for food (its seeds contain a significant amount of protein and fat oil) and for cattle forage;

however, the most demanded product is guar gum which is formed in the secondary endosperm of guar seeds. Natural gum is applied in the food-processing industry as a consistence stabilizer, increasing viscosity and enhancing the gelatinizing properties of the substance, as well as in cosmetology, paper, textile, coal and oil-drilling industries. The urgent necessity of guar gum import substitution, primarily, having an industrial purpose, as it is used at drilling of oil wells, has caused the actualization of the problem of the cultivation of this tropical crop in the climatic conditions of Russia.

Guar is not resistant to some fungal, bacterial, virus, nematode diseases and pests that periodically bring essential damage to the plant. In fact, the list of phytopathogens including obligate parasites and hemibiotrophs is rather extensive. The most harmful diseases of guar are *Alternaria* leaf blight caused by *Alternaria cyamopsidis* Rangaswami & Rao [1], and bacterial leaf blight caused by *Xanthomonas axonopodis* pv. *cyamopsidis* (Patel) Vauterin. So, yield loss in India owing to the distribution of *Alternaria* leaf blight on guar crops reaches 60% [2], and of bacterial leaf blight 68% [3]. Guar gall midges *Contarinia texana* (Felt) can destroy up to 30% of grain yield [4, 5], and is one of the most serious phytophages. Plant greenflies, thunder flies, frog-flies, white flies, Coleoptera pests [6-8] also bring a lot of harm. Thus, the necessity of the analysis of the Russian phytopathogenic landscape of all prospective regions for guar cultivation is obvious. The risk of damaging the new crop by pests-oligophages and widely specialised pathogens is especially great. The fungus *Fusarium solani* (Mart.) Sacc., causing wilt and root rot in India [9, 10], is propagated everywhere in Russia. Two diseases resulted in yield losses at experimental guar crops at the Ust-Labinsky District of the Krasnodar Territory in Russia – *Alternaria* leaf blight and bacterial rot [11].

The purpose of the present review is to summarize available data about guar interaction with harmful organisms, the plant resistance to the most dangerous pathogens and pests and the possibility of guar selection for the development of immunity to them.

**Resistance to bacteriosis.** The bacterial leaf blight agent of guar *X. axonopodis* pv. *cyamopsidis* was first revealed in two states of India in 1952 [12], and soon in the USA [13], Brazil [14], and other countries. The infection contamination is retained in seeds, which promotes the extensive and fast spread of the disease [15]. Epiphytotic development of the disease (the affection of plants reaches 80%) is usually observed after a long period of showers [16, 17].

The studies investigating the factors of plant resistance to the pathogen are not numerous. It was found out that forms susceptible to the pest (first of all, the PNB variety) show a decrease in the activity of peroxidases and polyphenol oxidases. The absence of such a decrease or an increase in the activity of these ferments can be used as a marker in case of selection of plants resistant to the disease [18]. Contamination of the resistant sample HG 75 resulted in an essential increase in the levels of phenols and peroxidases in plants [19]. Susceptible (Pusa Nav Bahar), moderately resistant (HG 563, FS 277) and highly resistant (wild progenitor *Cyamopsis serrata* Schinz.) guar samples were investigated for the content of solvable and structural (cellulose, hemicellulose, lignin) carbohydrates after artificial inoculation of plants with *X. axonopodis* pv. *cyamopsidis*. The maximum decrease in the concentration of solvable sugars was noted in inoculated plants of the susceptible sample; the sample *C. serrata* had a minimum change in this parameter. The susceptible variety also showed the decrease in the level of structural carbohydrates; on the contrary, the resistant forms demonstrated the raised level of carbohydrates as well, and that indicates the formation of protective barriers [20].

The resistance of the samples HG 75 and RGC 137 to the pathogen is controlled by dominant genes, which interact in a non-allelic way [21]. In F<sub>2</sub> from the crossing of the samples HG 75 and HG 563 with sensitive testers, phenotypes segregated as 13 resistant to 3 susceptible. The authors believe that both HG 75 and HG 563 have two key genes; one of them controls resistance to the disease, and the second one inhibits its development [22]. Thus, with equal probability, it is possible to assume that each of these samples possesses both dominant and recessive genes of resistance to bacteriosis. Unfortunately, genetic control of guar resistance to bacteriosis and other harmful organisms is discussed only in two small articles. The starting point of the research was the work [23] that supposes a high productive transcriptome (RNA-seq) sequencing of two guar varieties (M-83 and RGC-1066), carried out recently. In this work, there are 62146 unique coding sequences, deciphered and annotated, 5773 microsatellite (SSR) markers and 3594 mononucleotide polymorphisms (SNP) which are identified.

*X. axonopodis* pv. *cyamopsidis* is characterized by differential interaction with host-plant genotypes. In the USA, two strains of the pathogen (0 and 1) were distinguished, which differ not only in virulence to guar varieties [24], but also serologically [25]. A protocol of Enzyme-Linked Immunosorbent Assays (ELISA) is elaborated for detecting virulent and avirulent strains of the bacterium [26]. *X. axonopodis* pv. *cyamopsidis*, collected in the north and the north-west of India, essentially differed in aggressiveness when they were proliferated on resistant (HG 75) and sensitive (PNB) genotypes of guar. The analysis of the isolates with the use of molecular markers based on the polymerase chain reaction (first of all, RAPD) has also revealed significant polymorphism of the pathogen population. The results obtained using two different approaches correspond to each other [27].

Resistance to *Alternaria* leaf blight. The causative agent of *Alternaria* leaf blight was first revealed in 1953 in India [28], then in the USA [29] and other countries where guar was cultivated. The extensive spread of the disease was promoted by the fact that the infection contamination retained in seeds [30]. It was found out that the development of the fungus mycelium is optimal at a temperature of 35 °C [31]. Other experiments demonstrated the most severe development of the disease in the case of the variety Pusa Navbahar (PNB) at 25-31 °C, 80% of relative air humidity and heavy rainfall [32]. Monitoring of *A. cyamopsidis* on moderately resistant (HG-75, HG-365), moderately susceptible (RGC-1000), susceptible (RGC-936, RGC-1002) and highly susceptible (FS-277) varieties of guar has shown some regularity. The disease affects susceptible samples more intensively, and the degree of leaves lesion development depends, first of all, on insolation, the minimum air temperature, precipitation and relative air humidity in the evening [2, 33].

Guar resistance to the disease is not related with its anatomic and morphological features. The anatomical characteristics of leaves of two moderately resistant (HG-75, HG-365), four susceptible (HG-258, HFG-119, RGC-936, RGC-1017) and highly susceptible (FS-277) to *Alternaria* leaf blight guar varieties were compared. All samples did not differ in the number and size of stomata on both surfaces of leaf plates. The upper surface of the leaves in the case of moderate stable forms had a bit larger number of hairs [23-25], in comparison with susceptible samples [13-17], but the pubescence of the bottom surface of the leaf plate of all varieties did not differ much. The amount of wax on leaves of the stable forms at all growth stages was a bit exceeded, in comparison with susceptible samples; however, distinctions were not statistically significant [34].

Guar inoculation with *A. cyamopsidis* resulted in the essential accumula-

tion of polyphenol oxidase and phenolic compounds [35, 36]. U.N. Joshi et al. [30, 36], investigating the biochemical composition of the guar samples, susceptible (IC 116835) and moderately resistant (IC 116903) to the *Alternaria* leaf blight causative agent, revealed the increase in the activity of enzymes, and the accumulation of phenols and lignin in response to infestation by the pathogen. The plants of the resistant variety RGC-986 showed the highest level of solvable protein and phenolic compounds in comparison with moderately resistant (RGC-1003) and susceptible (RGC-936) forms. Infestation by the pathogen resulted in the most essential decrease in the solvable protein level in the case of the susceptible sample and the increase in the content of phenols in the case of the variety RGC-986. The infected plants of the resistant sample demonstrated the greatest concentration of sugars [37, 38].

**Resistance to other diseases.** Data on guar resistance to causative agents of other diseases note mainly the presence of nonspecific reactions of plants in response to pathogen contamination. The induced resistance was observed in the case of inoculation of guar samples with different resistance to the causative agent of charcoal rot *Macrophomina phaseolina* (Tassi) Goid. The leaves and roots of resistant forms revealed the highest activity of peroxidase and some other enzymes and accumulation of phenolic compounds. The variety RGC 1031 [39, 40] appeared to be the steadiest. The marker SCAR-20 has been developed which allows identifying the sample RGC 1031 [41]. In the case of infestation with the guar wilt causative agent *F. solani*, the plants demonstrated the decrease in the protein level and the rise of activity of proteolytic enzymes [10].

Phytopathologic and DNA testing showed that isolates of *M. phaseolina*, collected on guar and other plants, differ in specificity to plant hosts and aggressiveness when they are proliferated on the susceptible variety FS 277 [42].

Addition of zinc ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ), copper ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) and manganese ( $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ ) fertilizers into the soil induced the formation of resistance to root rot (causative agents *Rhizoctonia* spp.) in the susceptible sample FS 277, what testified to the decrease in the lesion degree of the infected plants. Thus, the plants revealed the raised activity of oxidative enzymes and accumulation of phenolic compounds and structural carbohydrates [43-45]. System resistance of guar to *Rhizoctonia solani* J.G. Kuhn was also induced by processing of seeds of the variety Local with salicylic acid and/or *Pseudomonas fluorescence* Migula. The accumulation of PR-protein (chitinase,  $\beta$ -1,3-glucanase), phenolic compounds and lignin, as well as an increase in the activity of enzymes, were revealed in sample plants [46].

In India and Pakistan, papaya (PaLCuV) and tomato (ToLCV) leafroll viruses, a yellow tomato leafroll virus (TYLCV), and a recombinant guar leafroll virus (CyTLCuV) which are proliferated by whitefly *Bemisia tabaci* Genn., invoke deformation of leaf plates, contraction of interstices and stems of guar plants [47-51]. In India, tobacco streak virus (TSV) is revealed in guar, which invokes mosaic and necrosis of leaves and necrotic strips on stems [52]. Bean common mosaic virus (BCMV) is well spread in guar crops, which is transmitted not only by greenflies but also with inoculated seeds [53].

**Resistance to insect pests.** Articles connected with guar resistance to insect pests are not numerous. S.P. Singh et al. [54] investigated 40 guar samples and revealed 3 precocious forms (HG 365, HG 563, RGC 1066), poorly colonized by greenfly *Aphis craccivora* (Koch). The greatest number of pests is marked on late-ripening varieties. Guar colonization with *A. craccivora* resulted in accumulation in plants of phenolic compounds and ferments, and a decrease in the level of carbohydrates and amino acids. It is curious that the maximum gathering of natural gum was received from the plants with the raised

level of carbohydrates and phenols that caused the raised resistance to the phytophage [55].

In result of the research of 60 guar varieties, 5 samples (CH 14-2, HG 75, HG 94, HG 258, HG 365), characterized by resistance to whitefly *B. tabaci*, were revealed [56, 57]. Eight guar samples were estimated for resistance to leaf beetles (family *Chrysomelidae* Latreille), leafhoppers *Amrasca biguttula biguttella* (Ishida), *Empoasca* Walsh spp., greenflies *Aphis medicaginis* Koch and American clover miners *Liriomyza trifolii* (Burgess). High-yielding samples RGC-1031 and GAUG-13 were less invaded by the phytophages [58]. It was reported about complex resistance of the guar variety BR-99 to insect pests (leafhopper, whitefly) and root rot in Pakistan [59].

**Breeding for resistance.** The literature data note low genetic diversity of sources of guar resistance to pathogens. In the field and laboratory experiments, it was proved that the variety Brooks is resistant to *A. cyamopsidis* and bacteriosis in the USA [29]. Among the varieties resistant to bacteriosis and *Alternaria* leaf blight, one can mention the varieties Hall and Mills, and their derivative forms, the Kinman, Esser, and the high-yielding variety Lewis as well [60]. The variety Lewis is selected in F<sub>8</sub> from the cross of the line T64001-12-1-B-3-2-B-2 (Brooks × Mills) with the sample PI 338780-B from India [61]; the variety Santa Cruz [62] has an identical ancestry. Cultivation of genetically homogeneous varieties accelerated adaptable microevolution of the pathogen. There were already reports that the varieties Brooks, Hall and Mills began to be severely affected by the causative agent of bacteriosis [63]. The lineage of the guar varieties resistant to diseases from India is not discussed in the literature. Besides the above-mentioned varieties, resistance to *Alternaria* leaf blight was revealed in samples HFG-14, HFG-236, HFG-516, HFG-522, HFG-530, HFG-554 [64], CVS, RGC-619, RGC-677 and RGC-679 [65], HG-182 [66]. It is reported about resistance to diseases of samples RGC 986, RGC 1003, RGC 1002, RGC 1017, RGM 112 [67]. Resistance to the anthracnose causative agent *Colletotrichum capsici* f. sp. *cyamopsicola* (Desai & Prasad) is noted for the variety RGC 673 [68].

The overwhelming majority of selection and genetic works has been carried out in India until now, where the most extensive collection of guar is put together (about 5 thousand accessions). Intraspecific crosses and phenotypic selection are most often applied. Guar has highly variable morphological traits [69, 70], but the diversity of its cultivated varieties for the genes of resistance to phytopathogens is low [71].

The most widespread harmful organism, the causative agent *X. axonopodis* pv. *cyamopsidis*, is shown to differentially interact with host-plant genotypes. The problem of overcoming of plants resistance due to the spreading of new intraspecific forms of harmful organisms is relevant in the case of other economically important varieties. It means that for the prevention of epiphytotics and mass reproduction of pests, it is necessary to grow varieties with different resistance genes. The prospective approach for dilating of genetic diversity is introgression of resistance genes from the wild varieties *C. senegalensis* Guill. & Perr. and *C. serrata* Schinz. [71]. There are also some means of population control of the harmful organisms based on management of plants populations in space and time: incorporation, strain-change, mosaics of species. Incorporation of different varieties of plants has been popular in India recently.

Guar cultivation together with companion crops essentially reduced the number of harmful pests (leafhoppers, whiteflies, greenflies) on plants. Therefore, at the use of millet as intercropping, the colonization of guar by *A. craccivora* has appeared to be the lowest, and the yield the highest [72].

Companion guar crops, which were located either near to okra *Abelmoschus esculentus* (L.) Moench or as a shelterbelt along the edge and inside the field, caused a decrease in the number of sucking and gnawing depredators of okra, and also attracted entomophages [73]. Intercropping of castor-oil plant (*Ricinus communis* L.) and guar in the ratio 2:1 also significantly reduced the number of harmful pests on castor-oil plant and involved useful entomofauna [74].

Phytosanitary monitoring of guar crops in Russia. In 2017, the authors carried out guar phytosanitary investigations (nurseries, collection study, and ecological testing) at the VIR Kuban experimental station (Gulkevichsky District, Krasnodar Territory) and the analysis of the infected vegetative material. In the beginning of July, the phytosanitary monitoring (shoots) showed the obvious domination of representatives of the family *Aphididae* (aphids) of the order *Homoptera* (homopterous) on juvenile plants. In all nurseries, the episode of a population explosion of black bean aphid *Aphis fabae* Scopoli was observed: the number of pests on some plants exceeded 2 thousand individuals on a propagule. The colonies of peach aphid *Myzus persicae* (Sulzer) and pea aphid *Acyrtosiphon pisum* Harris were also revealed. In the case of strong proliferation, the death of plants was noted. Predators were not found in aphids' colonies; the individuals mummified by parasites were rare. Spreading of the virus infection contamination, which was proliferated by aphids (yellowing and leaves marbling), began. The pathogenic mycoflora was represented mainly by the fungus species *Alternaria* Nees invoking *Alternaria* leaf blight. The beginning of spreading of bacterial spot was revealed. In the end of August (blooming-fructification), it was revealed that after insecticidal treatment there were only individual colonies of aphids on guar. After their mass reproduction, severe focal virus lesion of plants was observed. The analysis of rhizospheric pathogenic mycoflora demonstrated the domination of the fungus species *Verticillium* Nees and *Fusarium* Link. As during the first estimation, two general propagated diseases were revealed, the *Alternaria* leaf blight and bacteriosis; however, epiphytotic development was characteristic only for the last one. Mass wilting and death of plants of some samples were noted [75]. In three independent experiments, the collection guar samples were estimated in bacteriosis epiphytotics. The highest resistance to disease was revealed in k-52569 (Pakistan), k-52575 (USA) and k-52580 (India). Some forms were selected only in one of the experiments, which is probably conditioned by the heterogeneity of the samples. Apparently, lines resistant to the disease can be selected from the majority of collection samples [75].

Thus, the most harmful guar disease, in Russia as well, is bacteriosis. Sources of resistance to the pathogen are revealed in India and on the American continent, but the term of their use is circumscribed owing to the specificity of parasite-host interrelation. *Alternaria* leaf blight and vascular root rot are among the potentially dangerous diseases. Among phytophages, greenflies are the most harmful, as they are vectors of the viral infection. Infection by pathogens and colonization by phytophages induces defense response in guar plants. Because of differential interaction of harmful organisms with genotypes of a host, cultivation of genetically homogeneous varieties leads to mass reproduction of pests and diseases epiphytotics. Therefore, it is necessary to involve in the selection as many varieties as possible. In cultivation, it is better to alternate in time varieties with different resistance genes, to use mosaics (cultivation of many varieties with unequal resistance genes in the pathogen geographic range) and mixed varieties (the approach which was well proved against harmful pests). In breeding, it is perspective to create multilinear varieties (mechanical admixtures of phenotypic similar lines with unequal resistance genes) and pyramiding (merging of various

resistance factors in one genotype). It should be noticed that genetic researches of plant resistance and intraspecific variability of pathogens are still insufficient. In Russia, they became more dynamic within the frame of the project of creation of guar varieties with complex resistance (Vavilov All-Russian Institute of Plant Genetic Resources). In this program, the search for molecular markers and genes-candidates of economically valuable traits is parallel to sequencing allelic gene variants encoding guar resistance to diseases and pests.

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