

GAMMA RADIATION INDUCED MUTAGENESIS-SELECTION SYSTEM FOR SMUT RESISTANCE IN SUGARCANE

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(Manuscript received 19 December 2011)

Abstract

Sugarcane smut disease, caused by *Ustilago scitaminea* Syd. is universal in distribution being important in every sugarcane producing country including Egypt. The best control measure is the use of resistant cultivars developed by the usual breeding programmes or less common physical treatments as irradiation. In the present study, doses of Gama radiation (0.5, 1, 2, 3 kr) were used to induce mutagenesis in sugarcane buds of the commercial cultivar GT54-9. The normal and transformed percentage of smutted stools of GT54-9 mutants (257mutants) artificially inoculated (dipping and injection inoculation) with smut spores for 3 seasons were field evaluated. The mutants were grouped into 5 groups according to the transformed percentage of infection i.e, group 1 (0 -1%, 38 mutants), group 2 (>1-5%, 29 mutants), group 3 (>5-20%, 71 mutants), group 4 (>20-40%, 65 mutants), group 5 (>40%, 55 mutants). The untreated mother cultivar was placed in group 3 (5-20% infection). The results revealed the importance of direct mutagenic treatment and selection for improvement of commercial sugarcane varieties in Egypt.

Keywords : Gamma radiation , *Ustilago scitaminea*, Mutagenesis, Induced mutation, sugarcane, Smut.

INTRODUCTION

Sugarcane smut, caused by the Basidiomycetes fungus *Ustilago scitaminea* Syd. 1924 (*Sporisorium scitamineum* (Syd.) Piepenbring, *et. al.*,2002), is cosmopolitan in distribution and has been considered as an important disease in nearly every sugarcane producing country. It can reduce crop yields by more than 50% and make ratoon crops unprofitable to maintain. It is highly infectious and even developed countries have been unable to stay smut free with the use of appropriate quarantine measures. In Egypt the diseases was reported for the first time in 1930 and again in 1935 (Jones *et. al.*,1935). During 1982 and 1983, the disease was recognized on NCo-310 as sporadically distributed cases in Aswan, Qena and El-Menia governorates with infection incidence ranging between less than 1% up to 70% (El-Zayat *et. al.*,1986). The European and Mediterranean plant protection organization (EPPO) has rated smut

distribution in Egypt as widespread (CABI/EPPO, 2008). The best control method is the use of resistant cultivars. There is a strong genetic basis for resistance and resistant varieties have been readily available and used to control outbreaks of smut in several countries (Churchill *et. al.*,2006).

Sugarcane is a polyploid and highly heterozygous crop with wide variation in chromosome number, and is considered from the breeding point a difficult manipulated crop of view. Hybridization is generally practiced under controlled environment, which is a limiting factor in Egypt. Another way to obtain genetic variation is from somatic (bud) mutation either spontaneous or induced ones. Induced mutation, thus play a vital role in creating additional genetic variation. Normally a large plant population is required to raise segregation population (Rao, 1969).

For the past 80 years, mutation induction has been a routine tool for the generation of genetic variation in crop germplasm, and has been overwhelmingly used in crop improvement, a strategy that is known as Mutation Breeding. Since the establishment of the Joint FAO/IAEA Division of the Nuclear Techniques in Agriculture more than 3083 mutant varieties have been officially registered in the Database of mutant varieties and genetic stocks, Joint FAO/IAEA programme (Anon, 2010). Maluszynski *et. al.*,(2000) reported that more than 1585 mutant varieties have been officially released after a direct mutagenic treatment and selection in the subsequent generations. Gamma rays were employed to develop 64% of the radiation-induced mutant varieties, followed by X-rays (22%).

Induced mutations have allowed introduction of stable, desirable traits in different crops species (Takagi and Raham, 1996). Several breeders have reported the successful use of induced mutations for mosaic virus and smut disease resistance in sugarcane (Srivastava *et. al.*,1986). In the last five decades 13 mutants of sugarcane cultivars were registered and released in India, China, Cuba and Japan (Anon, 2010).

The present research work was conducted to induce mutation in sugarcane for smut resistance through the use of gamma rays induced mutation for the improvement of sugarcane.

MATERIALS AND METHODS

Plant material

Commercial sugarcane cultivar GT 54-9 obtained from Sugar Crops Research Institute ARC Egypt, was used in this work. This cultivar has been rated as a moderately resistant variety to sugarcane smut (El-Zayat *et. al.*1986).

Radiation treatment

Sugarcane stalks were stripped of all leaves, cut into 10 cm pieces using clean garden scissor, each piece contained one mature healthy look bud (eye) in the middle. Stalk pieces packed as 10 pieces/plastic bag (25x15 cm) as a preparation for the radiation process. Gamma rays were administered to a total 1248 buds of cultivar GT 54-9 at the National Centre for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority, Nasr city, Cairo, Egypt. The setts of each cultivar divide to four groups each group of cultivar GT 54-9 contained Approx. 330 bud to each dose. Dormant single bud setts of the two cultivars were given acute gamma radiation exposures of 0.5, 1.0, 2.0 and 3.0 kilorad (Kr). Each group of setts received one of the tested Gamma irradiation doses.

Cultivation of the gamma irradiated setts under greenhouse condition

The gamma irradiated sugarcane setts were moved to a private field in Meet Elkorashy, Dakahleia governorate to initiate a mutant bank.

Sugarcane Setts were given a hot-water treatment for 10 min at 52°C to stimulate growth and to assure the elimination of any pests or diseases. After hot-water treatment, the setts were dipped into 200 mg a.i./L fungicide solution Carboxin, C₁₂H₁₃NO₂S (**Vitavax**®) for few seconds and planted in 15 cm diameter pots filled with a mix of pitmos, sand and soil (1:1:1) in a greenhouse for germination. The plants irrigated every 2 to 3 days.

Transplanting and initiation of mutant bank under field condition

After two months in the greenhouse the pots of the germinated plants were transferred to the field bank and transplanted in plots. Each plot consisted of 5 rows 0.6 m apart and 6-m long and the distance between plants were 0.4 m. Each plant had a label showing mother cultivar name, radiation dose and code number. Recommended NPK fertilizers were added at rates of 210 kg N (as urea 46.5 % N), 45 kg P₂O₅ (as calcium super phosphate 15.5 % P₂O₅) and 48 kg K₂O (as potassium sulphate, 48 % K₂O)/fed. Phosphorus fertilizer was applied during seedbed preparation. Nitrogen and potassium fertilizers were added in two equal doses after two and three months from planting. The other agricultural practices were followed as recommended by the Sugar Crops Research Institute, Giza, Egypt.

Field screening of resistant and susceptible sugarcane mutants

After 9 months, mature seed cane from the first generation of mutated vegetative mutants from the field bank was harvested and each mutant stalks was warped together with a label indicating its identity. Sugarcane stalks of each mutant

were stripped, cut into one-budded setts. Sixty of the latter (10 cm cuts) were put together in one polyethylene Net-bag with the label as a preparation for the inoculation process with *U. scitaminea* spores.

Inoculation of sugarcane mutants with *U. scitaminea*

Preparation of the inoculum

Sugarcane smut whips collected from diseased varieties cultivated in sugarcane main growing areas in Upper Egypt (Quina, El-Minia and Asswan governorates) and from pre-released varieties from Sugarcane Research Station Mattana, Quina governorate one month before experimentation. The collected whips were separated and kept on a bench under room temperature for 5 days. After being dried, the whips homogenized in a big mortar to release the spores, then screened through a fine mesh to collect the spores. The collected spores bulked to form a composite mixture and the spores were tested for viability on potato dextrose agar. Spores stored in paper bags in the laboratory under dry conditions (Gillaspie *et. al.*,1983).

Inoculating sugarcane mutants

One-budded sets of sugarcane mutants in Net-bags were dipped in a suspension of smut spores (3g per liter of water) for 60 minutes and then held overnight on a layer of polythene sheets and covered with another layer (Gillaspie *et. al.*,1983).

Cultivating the inoculated mutants

This experiment was conducted in another field, (6 km far from the mutants field bank in Meet El-Korashy) in sand clay loam soils. Sixty of one-budded setts of each mutant were planted in 3 replicates in a complete randomize block design. Each replicate had 20 of the one-budded setts cultivated in 6 m long row spaced 30 cm. The rows spaced 50 cm apart and each row had a different mutant.

The recommended NPK fertilizers as mentioned before were added according to the recommendations of the Sugar Crops Research Institute. Manual weeding control was carried out in the entire field. Surface irrigation was employed in the field immediately after planting and thereafter with 7 to 14 days intervals depending on the weather temperatures during the season.

Second and third season (1st and 2nd ratoon)

After harvesting the first season sugarcane, the field cleaned from all the trash and dry leaves, irrigated and left for 4 weeks for the emergence of the new

plants from ratoon. The field fertilized with 80 Kg Nitrogen per feddan. Manual weeding control was carried out in the entire field. Surface irrigation was employed in the field immediately after planting and thereafter at 7 to 14 day intervals according to the prevailing weather temperatures during the season.

Inoculation using Injection method

The hypodermic injection technique according to Gillaspie *et. al.*,(1983) was used to re-inoculate the new emerged plants in the second and the third seasons. Shoots were inoculated when they were 20 cm long with 0.1 ml/injection around the meristemic region, or until the inoculum was forced out the shoot tip. The number of infected plants showing the typical symptoms of smut (whip formation) was recorded weekly for each mutant during the time of experiment. After the end of each season, the infected stool was then removed. The remaining Sugarcane plants harvested after nine months in the field. At the end of the third season, sugarcane was harvested and the trash burned in the field then being flooded with water for 2 weeks to eradicate any remains of smut inoculum in the field.

Data were recorded as a simple percentage of infection (presence or absence of a whip) of plants within each mutant and replication. Percentage were transformed by $\text{Sin}^{-1}y^{1/2}$ and expressed to degrees according to Burner *et. al.*,(1993), where y = infection percentage in decimal form. Zero percentage were equal to $\text{Sin}^{-1}(1/4n)^{1/2}$ where $n=20$ plants in 1st season, number of plants varied depending on the total percentage of infected plants in the 2nd and 3^{ed} seasons.

The number of infected plants showing the typical symptoms of smut (whip formation) were recorded weekly for each mutant during the time of experiment. The percentage of disease incidence were also converted to the sugarcane smut key of resistance according to Bailey and Bechet (1982) as follows: 0 to 1% highly resistant (HR), 1 to 5% (R) resistant, 5 to 20% intermediate (MR), 20 to 40% susceptible (S) and >40% highly susceptible (HS) (R.A., The infected stool was then removed. The remaining Sugarcane plants harvested after nine months in the field.

RESULTS AND DISCUSSIONS

A total of 1248 irradiated buds of sugarcane cultivars GT54-9 were grown in greenhouse (Table 1). The Pre-emergence mortality gamma radiation treated buds reached 786 buds. During the experiment sugarcane borer *Sesamia cretica*, attacked sugarcane mutants and caused a loss of 122 plants. The total survivor plants in the field after transplanting were 276 mutants. It is worthy to mention that, a delay in germination was noticed in plants treated with doses 2 and 3 Kr.

Data in table (1) state that, the highest percentage of plant mortality was recorded in the radiation treatments 0.5 Kr and 1 Kr (79.9 and 96.1 %). On the other hand, the lowest percentage of mortality were those the untreated buds (24%) and 2 Kr treatment (65.6).

Leathal and mutagenic effects of ionizing radiation result from incompletely or incorrectly repaired DNA lesions. Among these lesions, strand breaks are considered to be most important as they interrupt continuity and integrity of the double helix. An unrepaired single strand break in ssDNA, an unrepaired double strand break in dsDNA and crosslinking of DNA to itself or proteins has been shown to be responsible for the leathal effects of ionizing radiation (Ward, 1985).

Table 1. Mean of pre- and post- emergence mortality (Mort.) at 30 days after cultivation in greenhouse and survival at 3 months after transplanting in the field of sugarcane cultivar GT54-9 treated with 4 levels of gamma radiation.

Cultivar	Dose (Kr)	Greenhouse assessment				Field assessment		% of Mort.	
		Treated buds	Pre-emerg. Mort.	Post-emergence mortality		Total Mort.	Mort.		Total survival
				Borers	Unknown reasons				
	0	25	1	5	0	6	0	19	24.0
GT54-9	0.5	239	172	15	2	189	2	48	79.9
	1	330	222	56	15	293	24	13	96.1
	2	331	193	17	6	216	1	114	65.6
	3	323	198	29	11	238	3	82	74.6
	Total	1248	786	122	34	942	30	276	77.9

In sugarcane irradiation studies the results revealed variable effects. In this regard, Siddiqui *et. al.* (1976) reported that, 4.0 Kr as being lethal dose.

The normal and transformed percentage of smutted stools of GT54-9 mutants (257mutants) artificially inoculated by either dipping or injection with smut spores for 3 seasons, were used for the evaluation of resistance and susceptibility degrees of the mutants to the disease. Data in table (2) show that, the percentage of smutted stools varied significantly between the mutants. The data showed that, the mutants can be sorted in 5 groups according to the normal percentage of infection scale, "HR" the first group 1 (0 -1% infection, 38 mutants), "R" group 2 (>1-5% infection, 60 mutants), "MR" group 3 (>5-20% infection, 91 mutants), "S" group 4 (>20-40% infection, 56 mutants), "HS" group 5 (>40% infection, 13 mutants). It is worthy to mention that the untreated control recorded 8.4% infection and classified with group 3 as moderately resistant (MR). It was also noticed that, the transformed data of the percentage of infection shifted the resistance ranking of the tested mutants (Fig. 1).

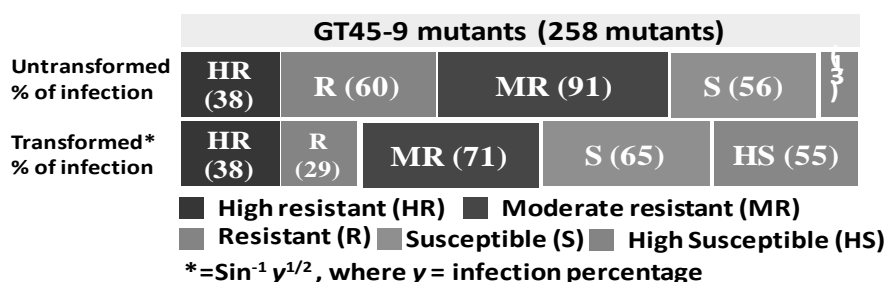


Fig.1. A diagram showing the shift in smut resistance rank according to the untransformed and transformed percentage of infection. of 258 mutants from sugarcane cultivar GT54-9, the numbers between practise showing number of mutants under each category.

This shift grouped the mutants to "HR" group 1 (0 -1% infection, 38 mutants), "R" group 2 (>1-5% infection, 29 mutants), "MR" group 3 (>5-20% infection, 71 mutants), "S" group 4 (>20-40% infection, 65 mutants), "HS" group 5 (>40% infection, 55 mutants) such differences between the two methods were reported by Burner *et. al.*,(1993). Data in table (2) show a high significant difference in the percentage of infection between sugarcane mutants affiliated to the different ranks of smut resistance. It also show that, the use of injection inoculation enhanced and increased the percentage of infection in some sugarcane mutants in the second and third seasons than the dipping inoculation in the first season.

Table 2. Number and Percentage of smutted stools of artificially smut-infected (by dipping one budded settes in spore suspension at 1st season and by hypodermic injection with spores in the 2nd and 3^{ed} seasons) sugarcane cultivar GT54-9 mutants (developed through gamma irradiation) under field conditions in micro plots.

Mutants	Percentage (%) of smutted stools					Transformed %				
	(untransformed)				Rank*	Transformed %				Rank
	1 st season	2 nd season	3 ^{ed} season	Total		1 st season	2 nd season	3 ^{ed} season	Total	
C9 CONT	6.7	1.7	0.0	8.4	MR	0.15	0.04	0.00	0.19	MR
C9/0.5Kr-1	0.0	1.7	1.7	3.3	R	0.00	0.04	0.04	0.08	MR
C9/0.5Kr-2	0.0	0.0	3.3	3.3	R	0.00	0.00	0.07	0.07	MR
C9/0.5Kr-3	6.7	7.2	0.0	13.3	MR	0.15	0.15	0.00	0.26	S
C9/0.5Kr-4	33.3	0.0	0.0	33.3	S	0.50	0.00	0.00	0.50	HS
C9/0.5Kr-5	0.0	0.0	3.3	3.3	R	0.00	0.00	0.07	0.07	MR
C9/0.5Kr-6	16.7	7.5	0.0	23.3	S	0.30	0.14	0.00	0.39	S
C9/0.5Kr-7	21.7	8.4	8.9	35.0	S	0.37	0.15	0.16	0.52	HS
C9/0.5Kr-8	0.0	0.0	1.7	1.7	R	0.00	0.00	0.04	0.04	R
C9/0.5Kr-9	33.3	0.0	0.0	33.3	S	0.50	0.00	0.00	0.50	HS
C9/0.5Kr-10	11.7	11.2	8.6	28.3	S	0.23	0.22	0.16	0.45	HS

Mutants	Percentage (%) of smutted stools (untransformed)					Transformed %				
	1 st	2 nd	3 ^{ed}	Rank*	Rank	1 st	2 nd	3 ^{ed}	Rank	
	season	season	season			Total	season	season		season
C9/0.5Kr-11	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/0.5Kr-12	0.0	0.0	1.7	1.7	R	0.00	0.00	0.04	0.04	R
C9/0.5Kr-13	0.0	8.3	3.5	11.7	MR	0.00	0.17	0.08	0.24	S
C9/0.5Kr-14	1.7	11.8	3.8	16.7	MR	0.04	0.24	0.08	0.31	S
C9/0.5Kr-15	0.0	1.7	1.7	3.3	R	0.00	0.04	0.04	0.08	MR
C9/0.5Kr-16	0.0	11.7	2.0	13.3	MR	0.00	0.24	0.04	0.26	S
C9/0.5Kr-17	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/0.5Kr-18	13.3	3.8	0.0	16.7	MR	0.26	0.08	0.00	0.31	S
C9/0.5Kr-19	0.0	0.0	3.3	3.3	R	0.00	0.00	0.08	0.08	MR
C9/0.5Kr-20	0.0	5.0	3.3	8.3	MR	0.00	0.11	0.07	0.18	MR
C9/0.5Kr-21	0.0	6.7	1.9	8.3	MR	0.00	0.14	0.04	0.17	MR
C9/0.5Kr-22	1.7	0.0	0.0	1.7	R	0.04	0.00	0.00	0.04	R
C9/0.5Kr-23	0.0	0.0	1.7	1.7	R	0.00	0.00	0.04	0.04	R
C9/0.5Kr-24	11.7	0.0	0.0	11.7	MR	0.24	0.00	0.00	0.24	S
C9/0.5Kr-25	5.0	0.0	1.8	6.7	MR	0.11	0.00	0.04	0.15	MR
C9/0.5Kr-26	3.3	17.4	1.9	21.7	S	0.08	0.31	0.04	0.37	S
C9/0.5Kr-27	5.0	0.0	1.7	6.7	MR	0.11	0.00	0.04	0.15	MR
C9/0.5Kr-28	3.3	0.0	3.3	6.7	MR	0.08	0.00	0.07	0.15	MR
C9/0.5Kr-29	21.7	10.4	0.0	30.0	S	0.37	0.18	0.00	0.47	HS
C9/0.5Kr-30	6.7	7.2	0.0	13.3	MR	0.15	0.15	0.00	0.25	S
C9/0.5Kr-31	3.3	5.2	0.0	8.3	MR	0.08	0.12	0.00	0.18	MR
C9/0.5Kr-32	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/0.5Kr-33	11.7	0.0	0.0	11.7	MR	0.24	0.00	0.00	0.24	S
C9/0.5Kr-34	0.0	0.0	3.3	3.3	R	0.00	0.00	0.07	0.07	MR
C9/0.5Kr-35	0.0	20.0	2.1	21.7	S	0.00	0.35	0.04	0.37	S
C9/0.5Kr-36	3.3	8.7	1.8	13.3	MR	0.08	0.18	0.04	0.26	S
C9/0.5Kr-37	5.0	12.3	0.0	16.7	MR	0.11	0.24	0.00	0.31	S
C9/0.5Kr-38	3.3	5.2	0.0	8.3	MR	0.08	0.12	0.00	0.18	MR
C9/0.5Kr-39	0.0	0.0	1.7	1.7	R	0.00	0.00	0.04	0.04	R
C9/0.5Kr-40	0.0	11.7	3.7	15.0	MR	0.00	0.24	0.07	0.28	S
C9/0.5Kr-41	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/0.5Kr-42	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/0.5Kr-43	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/0.5Kr-44	0.0	0.0	3.3	3.3	R	0.00	0.00	0.07	0.07	MR
C9/0.5Kr-45	5.0	3.5	0.0	8.3	MR	0.11	0.08	0.00	0.17	MR
C9/0.5Kr-46	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/0.5Kr-47	0.0	0.0	1.7	1.7	R	0.00	0.00	0.04	0.04	R
C9/0.5Kr-48	0.0	15.0	7.7	21.7	S	0.00	0.28	0.14	0.37	S
C9/1Kr-1	16.7	2.0	4.0	21.7	S	0.30	0.04	0.08	0.37	S
C9/1Kr-2	5.0	3.5	0.0	8.3	MR	0.11	0.08	0.00	0.17	MR
C9/1Kr-3	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/1Kr-4	10.0	15.0	6.7	28.3	S	0.20	0.27	0.13	0.44	HS

Mutants	Percentage (%) of smutted stools (untransformed)					Transformed %				
	1 st	2 nd	3 ^{ed}	Total	Rank*	1 st	2 nd	3 ^{ed}	Total	Rank
	season	season	season			season	season	season		
C9/1Kr-5	8.3	3.5	3.9	15.0	MR	0.17	0.08	0.08	0.28	S
C9/1Kr-6	16.7	22.9	10.3	41.7	HS	0.29	0.37	0.17	0.58	HS
C9/1Kr-7	1.7	5.1	3.7	10.0	MR	0.04	0.11	0.07	0.19	MR
C9/1Kr-8	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/1Kr-9	5.0	0.0	0.0	5.0	R	0.10	0.00	0.00	0.10	MR
C9/1Kr-10	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/1Kr-11	0.0	1.7	0.0	1.7	R	0.00	0.04	0.00	0.04	R
C9/1Kr-12	13.3	5.8	4.1	21.7	S	0.26	0.12	0.08	0.37	S
C9/1Kr-13	38.3	19.6	0.0	50.0	HS	0.55	0.31	0.00	0.67	HS
C9/2Kr-1	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/2Kr-2	6.7	3.7	2.1	11.7	MR	0.15	0.07	0.04	0.21	S
C9/2Kr-3	8.3	7.1	0.0	15.0	MR	0.18	0.14	0.00	0.28	S
C9/2Kr-4	36.7	7.0	2.8	43.3	HS	0.54	0.13	0.05	0.61	HS
C9/2Kr-5	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/2Kr-6	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/2Kr-7	0.0	11.7	0.0	11.7	MR	0.00	0.23	0.00	0.23	S
C9/2Kr-8	3.3	5.2	0.0	8.3	MR	0.08	0.12	0.00	0.18	MR
C9/2Kr-9	0.0	5.0	1.8	6.7	MR	0.00	0.11	0.04	0.15	MR
C9/2Kr-10	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/2Kr-11	1.7	0.0	0.0	1.7	R	0.04	0.00	0.00	0.04	R
C9/2Kr-12	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/2Kr-13	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/2Kr-14	5.0	7.0	2.0	13.3	MR	0.11	0.15	0.04	0.26	S
C9/2Kr-15	0.0	0.0	3.3	3.3	R	0.00	0.00	0.07	0.07	MR
C9/2Kr-16	6.7	28.6	2.6	35.0	S	0.15	0.45	0.05	0.52	HS
C9/2Kr-17	18.3	0.0	0.0	18.3	MR	0.33	0.00	0.00	0.33	S
C9/2Kr-18	10.0	0.0	0.0	10.0	MR	0.21	0.00	0.00	0.21	S
C9/2Kr-19	35.0	36.9	0.0	58.3	HS	0.52	0.51	0.00	0.76	HS
C9/2Kr-20	0.0	21.7	0.0	21.7	S	0.00	0.36	0.00	0.36	S
C9/2Kr-21	13.3	5.8	0.0	18.3	MR	0.26	0.12	0.00	0.33	S
C9/2Kr-22	23.3	0.0	2.2	25.0	S	0.39	0.00	0.04	0.41	HS
C9/2Kr-23	11.7	3.7	4.0	18.3	MR	0.24	0.07	0.08	0.33	S
C9/2Kr-24	33.3	7.1	0.0	38.3	S	0.50	0.12	0.00	0.55	HS
C9/2Kr-25	0.0	0.0	3.3	3.3	R	0.00	0.00	0.07	0.07	MR
C9/2Kr-26	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/2Kr-27	0.0	8.3	1.8	10.0	MR	0.00	0.18	0.04	0.21	S
C9/2Kr-28	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/2Kr-29	8.3	14.4	0.0	21.7	S	0.18	0.27	0.00	0.37	S
C9/2Kr-30	5.0	3.5	0.0	8.3	MR	0.11	0.08	0.00	0.18	MR
C9/2Kr-31	0.0	0.0	5.0	5.0	R	0.00	0.00	0.11	0.11	MR
C9/2Kr-32	5.0	33.2	13.3	45.0	HS	0.11	0.50	0.23	0.62	HS
C9/2Kr-33	13.3	17.4	4.8	31.7	S	0.26	0.31	0.09	0.48	HS

Mutants	Percentage (%) of smutted stools (untransformed)					Transformed %				
	1 st	2 nd	3 ^{ed}	Rank*	Rank	1 st	2 nd	3 ^{ed}	Rank	
	season	season	season			Total	season	season		season
C9/2Kr-34	8.3	23.7	10.0	36.7	S	0.16	0.39	0.18	0.54	HS
C9/2Kr-35	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/2Kr-36	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/2Kr-37	0.0	18.3	0.0	18.3	MR	0.00	0.33	0.00	0.33	S
C9/2Kr-38	20.0	18.8	0.0	35.0	S	0.35	0.32	0.00	0.52	HS
C9/2Kr-39	0.0	8.3	0.0	8.3	MR	0.00	0.18	0.00	0.18	MR
C9/2Kr-40	15.0	0.0	1.8	16.7	MR	0.27	0.00	0.04	0.30	S
C9/2Kr-41	16.7	10.2	0.0	25.0	S	0.30	0.20	0.00	0.41	HS
C9/2Kr-42	8.3	0.0	0.0	8.3	MR	0.18	0.00	0.00	0.18	MR
C9/2Kr-43	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/2Kr-44	6.7	0.0	0.0	6.7	MR	0.14	0.00	0.00	0.14	MR
C9/2Kr-45	5.0	0.0	3.5	8.3	MR	0.11	0.00	0.07	0.17	MR
C9/2Kr-46	0.0	10.0	3.9	13.3	MR	0.00	0.20	0.08	0.25	S
C9/2Kr-47	0.0	30.0	6.7	35.0	S	0.00	0.47	0.13	0.52	HS
C9/2Kr-48	3.3	0.0	0.0	3.3	R	0.08	0.00	0.00	0.08	MR
C9/2Kr-49	0.0	0.0	1.7	1.7	R	0.00	0.00	0.04	0.04	R
C9/2Kr-50	0.0	8.3	0.0	8.3	MR	0.00	0.18	0.00	0.18	MR
C9/2Kr-51	6.7	0.0	3.4	10.0	MR	0.13	0.00	0.08	0.20	MR
C9/2Kr-52	3.3	5.2	1.9	10.0	MR	0.07	0.11	0.04	0.19	MR
C9/2Kr-53	0.0	13.3	4.2	16.7	MR	0.00	0.26	0.08	0.30	S
C9/2Kr-54	0.0	8.3	5.5	13.3	MR	0.00	0.17	0.12	0.25	S
C9/2Kr-55	23.3	21.5	0.0	40.0	S	0.39	0.35	0.00	0.57	HS
C9/2Kr-56	1.7	3.4	3.6	8.3	MR	0.04	0.08	0.08	0.17	MR
C9/2Kr-57	8.3	0.0	0.0	8.3	MR	0.17	0.00	0.00	0.17	MR
C9/2Kr-58	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/2Kr-59	0.0	0.0	3.3	3.3	R	0.00	0.00	0.08	0.08	MR
C9/2Kr-60	23.3	8.9	0.0	30.0	S	0.39	0.14	0.00	0.46	HS
C9/2Kr-61	0.0	0.0	5.0	5.0	R	0.00	0.00	0.11	0.11	MR
C9/2Kr-62	5.0	15.8	4.2	23.3	S	0.11	0.29	0.08	0.39	S
C9/2Kr-63	36.7	0.0	0.0	36.7	S	0.54	0.00	0.00	0.54	HS
C9/2Kr-64	0.0	0.0	1.7	1.7	R	0.00	0.00	0.04	0.04	R
C9/2Kr-65	0.0	0.0	1.7	1.7	R	0.00	0.00	0.04	0.04	R
C9/2Kr-66	0.0	6.7	0.0	6.7	MR	0.00	0.13	0.00	0.13	MR
C9/2Kr-67	0.0	0.0	1.7	1.7	R	0.00	0.00	0.04	0.04	R
C9/2Kr-68	35.0	0.0	0.0	35.0	S	0.52	0.00	0.00	0.52	HS
C9/2Kr-69	0.0	0.0	1.7	1.7	R	0.00	0.00	0.04	0.04	R
C9/2Kr-70	15.0	13.7	2.2	28.3	S	0.29	0.26	0.04	0.45	HS
C9/2Kr-71	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/2Kr-72	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/2Kr-73	0.0	0.0	1.7	1.7	R	0.00	0.00	0.04	0.04	R
C9/2Kr-74	3.3	5.4	3.4	11.7	MR	0.07	0.11	0.08	0.23	S
C9/2Kr-75	11.7	1.9	0.0	13.3	MR	0.24	0.04	0.00	0.26	S

Mutants	Percentage (%) of smutted stools (untransformed)					Transformed %				
	1 st	2 nd	3 ^{ed}	Total	Rank*	1 st	2 nd	3 ^{ed}	Total	Rank
	season	season	season			season	season	season		
C9/2Kr-76	0.0	1.7	0.0	1.7	R	0.00	0.04	0.00	0.04	R
C9/2Kr-77	20.0	2.0	0.0	21.7	S	0.35	0.04	0.00	0.37	S
C9/2Kr-78	0.0	1.7	0.0	1.7	R	0.00	0.04	0.00	0.04	R
C9/2Kr-79	0.0	1.7	3.4	5.0	R	0.00	0.04	0.08	0.11	MR
C9/2Kr-80	5.0	8.8	0.0	13.3	MR	0.11	0.18	0.00	0.26	S
C9/2Kr-81	0.0	5.0	0.0	5.0	R	0.00	0.10	0.00	0.10	MR
C9/2Kr-82	3.3	0.0	0.0	3.3	R	0.07	0.00	0.00	0.07	MR
C9/2Kr-83	0.0	0.0	5.0	5.0	R	0.00	0.00	0.11	0.11	MR
C9/2Kr-84	11.7	3.7	0.0	15.0	MR	0.23	0.08	0.00	0.28	S
C9/2Kr-85	0.0	5.0	0.0	5.0	R	0.00	0.10	0.00	0.10	MR
C9/2Kr-86	0.0	0.0	1.7	1.7	R	0.00	0.00	0.04	0.04	R
C9/2Kr-87	8.3	3.5	0.0	11.7	MR	0.17	0.08	0.00	0.23	S
C9/2Kr-88	0.0	1.7	0.0	1.7	R	0.00	0.04	0.00	0.04	R
C9/2Kr-89	0.0	0.0	1.7	1.7	R	0.00	0.00	0.04	0.04	R
C9/2Kr-90	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/2Kr-91	21.7	25.3	0.0	41.7	HS	0.36	0.40	0.00	0.59	HS
C9/2Kr-92	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/2Kr-93	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/2Kr-94	0.0	1.7	5.1	6.7	MR	0.00	0.04	0.11	0.14	MR
C9/2Kr-95	16.7	15.3	0.0	30.0	S	0.29	0.27	0.00	0.47	HS
C9/2Kr-96	20.0	20.8	0.0	36.7	S	0.35	0.34	0.00	0.54	HS
C9/2Kr-97	0.0	6.7	0.0	6.7	MR	0.00	0.12	0.00	0.12	MR
C9/2Kr-98	5.0	1.7	0.0	6.7	MR	0.11	0.04	0.00	0.15	MR
C9/2Kr-99	18.3	2.1	0.0	20.0	MR	0.32	0.04	0.00	0.34	S
C9/2Kr-100	0.0	3.3	0.0	3.3	R	0.00	0.08	0.00	0.08	MR
C9/2Kr-101	0.0	5.0	0.0	5.0	R	0.00	0.11	0.00	0.11	MR
C9/2Kr-102	23.3	11.0	0.0	31.7	S	0.39	0.21	0.00	0.49	HS
C9/2Kr-103	0.0	3.3	1.7	5.0	R	0.00	0.07	0.04	0.11	MR
C9/2Kr-104	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/2Kr-105	25.0	4.6	0.0	28.3	S	0.40	0.09	0.00	0.44	HS
C9/2Kr-106	0.0	3.3	0.0	3.3	R	0.00	0.07	0.00	0.07	MR
C9/2Kr-107	0.0	0.0	1.7	1.7	R	0.00	0.00	0.04	0.04	R
C9/2Kr-108	11.7	0.0	0.0	11.7	MR	0.24	0.00	0.00	0.24	S
C9/2Kr-109	1.7	0.0	0.0	1.7	R	0.04	0.00	0.00	0.04	R
C9/2Kr-110	0.0	3.3	0.0	3.3	R	0.00	0.08	0.00	0.08	MR
C9/2Kr-111	0.0	1.7	0.0	1.7	R	0.00	0.04	0.00	0.04	R
C9/2Kr-112	0.0	3.3	5.1	8.3	MR	0.00	0.08	0.11	0.18	MR
C9/2Kr-113	5.0	8.7	0.0	13.3	MR	0.11	0.18	0.00	0.26	S
C9/2Kr-114	10.0	5.6	0.0	15.0	MR	0.21	0.10	0.00	0.28	S
C9/3Kr-1	0.0	1.7	0.0	1.7	R	0.00	0.04	0.00	0.04	R
C9/3Kr-2	0.0	3.3	0.0	3.3	R	0.00	0.07	0.00	0.07	MR
C9/3Kr-3	13.3	0.0	0.0	13.3	MR	0.26	0.00	0.00	0.26	S

Mutants	Percentage (%) of smutted stools (untransformed)					Transformed %				
	1 st	2 nd	3 ^{ed}	Total	Rank*	1 st	2 nd	3 ^{ed}	Total	Rank
	season	season	season			season	season	season		
C9/3Kr-4	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/3Kr-5	6.7	0.0	0.0	6.7	MR	0.14	0.00	0.00	0.14	MR
C9/3Kr-6	26.7	13.8	0.0	36.7	S	0.43	0.23	0.00	0.54	HS
C9/3Kr-7	0.0	11.7	1.7	13.3	MR	0.00	0.17	0.04	0.21	S
C9/3Kr-8	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/3Kr-9	8.3	1.9	0.0	10.0	MR	0.18	0.04	0.00	0.20	MR
C9/3Kr-10	0.0	1.7	0.0	1.7	R	0.00	0.04	0.00	0.04	R
C9/3Kr-11	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/3Kr-12	0.0	0.0	1.7	1.7	R	0.00	0.00	0.04	0.04	R
C9/3Kr-13	10.0	0.0	3.7	13.3	MR	0.20	0.00	0.08	0.26	S
C9/3Kr-14	5.0	1.7	0.0	6.7	MR	0.11	0.04	0.00	0.15	MR
C9/3Kr-15	31.7	0.0	0.0	31.7	S	0.49	0.00	0.00	0.49	HS
C9/3Kr-16	25.0	0.0	0.0	25.0	S	0.41	0.00	0.00	0.41	HS
C9/3Kr-17	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/3Kr-18	0.0	6.7	0.0	6.7	MR	0.00	0.13	0.00	0.13	MR
C9/3Kr-19	0.0	5.0	5.0	10.0	MR	0.00	0.10	0.11	0.20	MR
C9/3Kr-20	0.0	6.7	0.0	6.7	MR	0.00	0.14	0.00	0.14	MR
C9/3Kr-21	20.0	6.1	0.0	25.0	S	0.35	0.12	0.00	0.41	HS
C9/3Kr-22	0.0	3.3	0.0	3.3	R	0.00	0.07	0.00	0.07	MR
C9/3Kr-23	6.7	0.0	0.0	6.7	MR	0.14	0.00	0.00	0.14	MR
C9/3Kr-24	13.3	0.0	0.0	13.3	MR	0.26	0.00	0.00	0.26	S
C9/3Kr-25	33.3	7.2	2.4	40.0	S	0.50	0.13	0.05	0.57	HS
C9/3Kr-26	18.3	4.4	0.0	21.7	S	0.33	0.08	0.00	0.36	S
C9/3Kr-27	0.0	5.0	1.7	6.7	MR	0.00	0.11	0.04	0.15	MR
C9/3Kr-28	11.7	11.4	8.4	28.3	S	0.23	0.22	0.16	0.45	HS
C9/3Kr-29	18.3	5.9	0.0	23.3	S	0.33	0.10	0.00	0.39	S
C9/3Kr-30	0.0	13.3	5.6	18.3	MR	0.00	0.23	0.11	0.32	S
C9/3Kr-31	0.0	8.3	0.0	8.3	MR	0.00	0.14	0.00	0.14	MR
C9/3Kr-32	0.0	5.0	0.0	5.0	R	0.00	0.11	0.00	0.11	MR
C9/3Kr-33	0.0	16.7	1.9	18.3	MR	0.00	0.30	0.04	0.32	S
C9/3Kr-34	28.3	7.7	0.0	33.3	S	0.45	0.12	0.00	0.50	HS
C9/3Kr-35	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/3Kr-36	15.0	0.0	0.0	15.0	MR	0.28	0.00	0.00	0.28	S
C9/3Kr-37	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/3Kr-38	45.0	16.7	0.0	55.0	HS	0.62	0.21	0.00	0.73	HS
C9/3Kr-39	0.0	1.7	0.0	1.7	R	0.00	0.04	0.00	0.04	R
C9/3Kr-40	6.7	7.1	0.0	13.3	MR	0.15	0.15	0.00	0.26	S
C9/3Kr-41	0.0	21.7	10.5	30.0	S	0.00	0.36	0.20	0.47	HS
C9/3Kr-42	0.0	0.0	3.3	3.3	R	0.00	0.00	0.07	0.07	MR
C9/3Kr-43	0.0	18.3	6.1	23.3	S	0.00	0.29	0.12	0.37	S
C9/3Kr-44	13.3	19.3	0.0	30.0	S	0.25	0.31	0.00	0.46	HS
C9/3Kr-45	31.7	25.1	3.3	50.0	HS	0.48	0.39	0.05	0.67	HS

Mutants	Percentage (%) of smutted stools (untransformed)				Rank*	Transformed %				
	1 st	2 nd	3 ^{ed}	Total		1 st	2 nd	3 ^{ed}	Total	Rank
	season	season	season			season	season	season		
C9/3Kr-46	21.7	4.2	0.0	25.0	S	0.37	0.08	0.00	0.41	HS
C9/3Kr-47	30.0	17.9	0.0	41.7	HS	0.47	0.23	0.00	0.58	HS
C9/3Kr-48	21.7	4.2	0.0	25.0	S	0.37	0.08	0.00	0.41	HS
C9/3Kr-49	0.0	13.3	4.2	16.7	MR	0.00	0.23	0.09	0.27	S
C9/3Kr-50	10.0	7.7	1.8	18.3	MR	0.19	0.15	0.04	0.32	S
C9/3Kr-51	26.7	4.6	2.2	31.7	S	0.43	0.09	0.04	0.49	HS
C9/3Kr-52	16.7	27.7	2.8	41.7	HS	0.31	0.42	0.05	0.59	HS
C9/3Kr-53	5.0	10.5	0.0	15.0	MR	0.11	0.19	0.00	0.27	S
C9/3Kr-54	3.3	1.7	0.0	5.0	R	0.07	0.04	0.00	0.11	MR
C9/3Kr-55	11.7	1.8	7.7	20.0	MR	0.23	0.04	0.16	0.35	S
C9/3Kr-56	35.0	10.3	0.0	41.7	HS	0.52	0.15	0.00	0.59	HS
C9/3Kr-57	18.3	16.5	4.7	35.0	S	0.32	0.29	0.09	0.52	HS
C9/3Kr-58	10.0	11.3	0.0	20.0	MR	0.20	0.22	0.00	0.34	S
C9/3Kr-59	15.0	15.7	2.2	30.0	S	0.29	0.27	0.04	0.46	HS
C9/3Kr-60	0.0	18.3	0.0	18.3	MR	0.00	0.32	0.00	0.32	S
C9/3Kr-61	23.3	15.6	0.0	35.0	S	0.39	0.21	0.00	0.51	HS
C9/3Kr-62	0.0	1.7	0.0	1.7	R	0.00	0.04	0.00	0.04	R
C9/3Kr-63	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/3Kr-64	1.7	1.7	0.0	3.3	R	0.04	0.04	0.00	0.08	MR
C9/3Kr-65	11.7	0.0	0.0	11.7	MR	0.23	0.00	0.00	0.23	S
C9/3Kr-66	0.0	0.0	1.7	1.7	R	0.00	0.00	0.04	0.04	R
C9/3Kr-67	3.3	0.0	0.0	3.3	R	0.08	0.00	0.00	0.08	MR
C9/3Kr-68	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/3Kr-69	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/3Kr-70	31.7	12.1	2.8	41.7	HS	0.49	0.22	0.05	0.59	HS
C9/3Kr-71	18.3	11.8	2.6	30.0	S	0.32	0.21	0.05	0.46	HS
C9/3Kr-72	8.3	5.3	0.0	13.3	MR	0.16	0.10	0.00	0.23	S
C9/3Kr-73	0.0	0.0	0.0	0.0	HR	0.00	0.00	0.00	0.00	HR
C9/3Kr-74	3.3	5.2	0.0	8.3	MR	0.08	0.11	0.00	0.17	MR
C9/3Kr-75	10.0	7.6	6.1	21.7	S	0.20	0.16	0.13	0.37	S
C9/3Kr-76	18.3	10.0	0.0	26.7	S	0.32	0.20	0.00	0.43	HS
C9/3Kr-77	33.3	10.3	0.0	40.0	S	0.50	0.15	0.00	0.57	HS
C9/3Kr-78	1.7	0.0	0.0	1.7	R	0.04	0.00	0.00	0.04	R
C9/3Kr-79	28.3	26.6	0.0	46.7	HS	0.45	0.39	0.00	0.64	HS
C9/3Kr-80	20.0	20.9	0.0	36.7	S	0.35	0.35	0.00	0.54	HS
C9/3Kr-81	0.0	8.3	0.0	8.3	MR	0.00	0.14	0.00	0.14	MR
C9/3Kr-82	6.7	1.9	0.0	8.3	MR	0.14	0.04	0.00	0.17	MR
LSD (0.05)	6.97	10.55	4.7	11.29		0.10	0.17	0.09	0.15	
CV %	49.07	63.31	67.16	42.82		49.07	63.31	67.63	42.82	

% = $\sin^{-1} y^{1/2}$, where y = infection percentage

CV%= Coefficient of Variation

Rank of smut resistance according to Bailey and Bechet (1982)

From the results of injection inoculation, the data confirmed that the inoculation procedure influenced the smut reaction of some mutants. Some mutants expressed a significant low percentage of infection in the dipping methods than the other mutants while when the injection method used, some of those mutants showed a significant low percentage in infection with the dipping method expressed a high significant infection percentage when injected with the spores. These results are in agreement with Miller *et. al.*,(1982), they reported that, the percentage of infection in some sugarcane clones increased from 7.6% in dipping inoculation to 70% in injection inoculation. On the other hand, these results would also differentiate between mutants with structural resistance and the others with physiological resistance.

Smut resistance in sugarcane is influenced by nodal bud morphology, chemical inhibitors present in bud scales and host physiology. Teliospore injection circumvents the protection afforded by intact bud scales and provides an estimate of physiological resistance to fungal development in the plant. Injection inoculation may induce greater smut infection than dip inoculation, and cultivars can respond differently to the two methods of inoculation (Olweny *et. al.*,2008).

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الإستحثاث الطفوري لأشعة جاما كنظام لإنتخاب قصب السكر المقاوم لمرض التفحم

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يعتبر مرض تفحم قصب السكر المتسبب عن الفطر *يوستيلاجو سيتامينيا* من أهم الأمراض في مصر و التي تنتشر في كل بلاد العالم المنتجه لقصب السكر. و تعتبر الأصناف المقاومه هي الطريقه الفعاله الوحيدة لمقاومة المرض. تم معاملة براعم الصنف التجاري GT54-9 بأربعة جرعات من أشعة جاما (0.5 و 1 و 2 و 3 كيلوراد) لإستحثاث التطفير. تم حساب النسب الطبيعيه و المحوله للإصابة بالتفحم في عدد 257 طفره بعد إجراء العدوي بواسطة غمر البراعم في معلق الجراثيم و حقن النباتات بمعلق الجراثيم لثلاثة مواسم متعاقبه. و قد أظهرت النتائج أنه يمكن تقسيم الطفرات الي 5 مجاميع حسب النسب المعدله للإصابة بالتفحم، المجموعه الأولي (0-1%، 38 طفره)، المجموعه الثانيه (<1-5% ، 29 طفره) ، المجموعه الثالثه (<5-20%، 71 طفره) ، المجموعه الرابعه (<20-40% ، 65 طفره) و المجموعه الخامسه (<40%، 55 طفره) في حين إنضم الصنف الأم الغير معامل للمجموعه الثالثه. و تعكس النتائج المتحصل عليها أهميه دور التطفير المباشر و الإنتخاب للحصول علي اصناف قصب مقاومه لمرض التفحم علاوه علي ان التطفير وسيله فعاله لإنتاج اختلافات وراثيه يمكن استخدامها في برامج التربيه.