Global Advanced Research Journal of Agricultural Science (ISSN: 2315-5094) Vol. 2(9) pp. 246-251, September, 2013.
Available online http://garj.org/garjas/index.htm

Full Length Research Paper

Methods for the screening of sorghum germplasm against sorghum head and loose smuts in Nigeria

*** Kutama[1], A.S., [2]Umar, S., [2]Binta, U.B. and [2]Tijjani, A.**

[1]Department of Biological Sciences, Faculty of Science, Federal University, Dutse, Jigawa state, Nigeria
[2]Department of Biological Sciences, College of Arts, Sciences and Remedial Studies, Kano

Accepted 30 September, 2013

Five artificial inoculation techniques which includes, stem injection, seed, soil, PDA-seedling, and cotton wool-seedling inoculation techniques were evaluated to determine the incidence and severity of sorghum head and loose smuts in two sorghum genotypes: SSV2006002 and SSV2008089 under field conditions for two growing seasons (2009 and 2010). The trials were carried out at the teaching and research farm of Bayero University, Kano, Nigeria, and laid out in a split plot design in which the sorghum genotypes occupied the main plots while inoculation techniques were the sub plots. Disease incidence varied significantly (P<0.05) between the different inoculation techniques and sorghum genotypes. For head smut, stem injection >PDA>, soil > cotton was the order of decreasing % disease incidence. No head smut infection occurred following seed inoculation in both the sorghum genotypes, of which SSV2008089 was more susceptible than SSV2006002. For loose smut, stem injection> seed > soil > PDA> cotton was the order of decreasing % disease incidence. So, all the techniques produced some diseased plants, with significant (P<0.05) difference between the inoculation techniques. Similarly, SSV2008089 had the higher disease incidence than SSV2006002. The severity also followed the same trend. Injection method produced most severely infected sorghum genotypes for both head and loose smuts with significant difference (P<0.05). Therefore, all the five artificial inoculation methods could be used to screen sorghum germplasm against head and loose smuts.

Keywords: inoculation methods, sorghum genotypes, head and loose smuts, disease incidence and severity

INTRODUCTION

Sorghum (*Sorghum bicolor* (L) Monarch) is one of the world's major food crops, particularly in areas of high temperature and low rainfall. Global production is estimated to exceed 40 million hectares, ranking it fifth in importance among cereals (FAO, 2012). The yield from sorghum is potentially very high (Fisher and Wilson, 1975) and can average 4, 505 kg ha^{-1} as that obtained in the USA (FAO, 1992). However, average yields in Africa have been estimated at 803 kg ha^{-1} to a maximum of 1.3 tones/ha in some areas in Nigeria (Marley *et al*., 2002a) which is the lowest globally and is tending to decline (Dogget, 1988). The reasons for this are that resource poor, small holder farmers do not have recourse to external inputs, statutory support, efficient communications or markets, presence of pests, parasites and diseases. Like all crops, sorghum is subject to terrible infectious diseases

*Corresponding Author's Email: kutamasak@yahoo.com; Tel: +2347067371893

which sometimes limit production. One of these destructive diseases is smut. In Nigeria, most of the sorghum varieties cultivated are mainly local land races and some few exotic or improved varieties that completely lack satisfactory resistance to diseases including smuts and Anthracnose (Gwary and Asala, 2006; Kutama *et al*., 2011a, b,c,d; Kutama, 2012, Kutama *et al*.,2013). Perhaps, only very few or no sorghum varieties are resistant to smut diseases in Nigeria and the world over (IPM, 2008; Kutama, 2012). Therefore, there is the apparent quest for continuous evaluation of these local varieties in sorghum growing areas of Nigeria for use by resource poor farmers and sorghum breeders. Use of resistant variety is and remains the only best strategy for the control of smut and many sorghum diseases in Nigeria (Gwary *et al*., 2007; Kutama *et al*., 2011a, b,c,d; Kutama, 2012, Kutama *et al*.,2013).

Many artificial inoculation techniques for the screening of sorghum genotypes against both loose and head smuts have been suggested by many workers (Sundaram, 1980; Kutama *et al*., 2011a,b; Kutama *et al*.,2013). It is however apparent that the appropriate inoculation procedure depends on the route of infection of a particular pathogen and the developmental stage of the host plant when it is most susceptible. These plus the question of whether small or large number of sorghum lines are used for the inoculation; partly decide which method mostly fit (Kutama, 2012). Earlier on, Claflin and Ramundo (1996) reported that the protocols used to screen for resistance to smuts are somewhat inconsistent because escapes are very common. Sorghum genotypes susceptible to smuts can easily escape disease due to the uneven distribution of infective propagules or spores of the smut pathogen in soil, air and seed or environmental factors that prevent spore germination or seedling infection. Inoculation with sporidia suspension is more reliable but sensitive to poor technical skills and genetic heterogeneity of the plant material (Osorio and Frederiksen, 1998), and could be tedious in large sorghum collections (Kutama *et al*., 2011a,b,c; and Kutama,2102). The objective of this work is to assess different artificial inoculation techniques used for the screening of sorghum genotypes against head and loose smuts.

MATERIAL AND METHODS

Evaluation of Effect of Inoculation Methods on Incidence and Severity of Head and Loose Smuts of Sorghum

The objective of this study was to assess some inoculation techniques on the resultant incidence and severity of the two smuts under study. In this experiment, five different inoculation techniques were assessed. In all cases, except where otherwise stated, the experiment was laid out in a split plot design in which the sorghum genotypes occupied the main plots while inoculation techniques were the sub plot. The plot size in all cases was 1.5 m by three rows. Sowing was done at the rate of three seeds per hole and a spacing of 45 cm between stands. NPK, (20:10:10) fertilizer was applied at the rate of 50 kg/ha in two split doses; two weeks after germination and at booting stage. The trials were conducted on research farm of the Faculty of Agriculture, Bayero University, Kano in 2009 and 2010. In both trials, two sorghum genotypes were used throughout, they were SSV2006002 and SSV2008089. The various inoculation techniques evaluated were;

(i) Injection of teliospores into stem

Two sorghum varieties were sown as mentioned above. Three weeks after sowing (3WAS), 0.5 g of previously collected, dried and stored teliospores of *S. cruentum* or *S. reilianum* was germinated separately in 1 liter of distilled water for 28 hours and blended for 30 seconds using an electric blender. One milliliter of the suspension was introduced into the main stem of the plant with a pediatric syringe by inserting the needle gently into the stem while carefully holding and supporting the whole plant with a hand to prevent damage. The same procedure was repeated 40 (DAS). The plants in both cases were allowed to grow normally up to physiological maturity Kutama *et al*.,2011 a; 2013,. A check was also established in the same manner but injected with sterile distilled water in place of teliospore suspension.

(ii) Mixing dry teliospores with seeds before sowing (Sundaram, 1980)

Seeds of each sorghum variety were mixed thoroughly with dry teliospores of S. cruenta or *S. reilianum* in the ratio of 1 g of spore to 1 kg of seed. The seeds together with some adhering spores were then sown at the rate of 3-5 seeds per hole. Following thinning, the plants (1/stand) were allowed to grow to physiologic maturity. A check consisted of plot sown with seeds that were not mixed with smut spores.

(iii) Sowing seeds in soil infested with teliospores

Seeds were sown in a 1 - 2 cm wide and 5 - 7 cm deep holes containing 0.2 - 0.5 g of teliospore of either *S. cruentum* or *S. reilianum*. Prior to sowing, a mixture of fine sandy loam soil obtained from the farm, and teliospore of *S. cruentum* or *S. reilianum* was made in the ratio of 50:1, soil: teliospore. The mixture was then used to cover the seeds in each hole. The check treatments consisted of plots grown from seeds sown in non-infested soil (Kutama *et al*., 2011b).

Table I. Effect of inoculation techniques on the incidence of infection of head smut in two sorghum genotypes

Inoculation technique	Sorghum head smut incidence (%) in two genotypes:	
	SSV2006002	**SSV2008089**
1. Mixing dry teliospores with seeds before sowing	0	0
2. Injecting teliospores into stem	62.5	87.5
3. Sowing seeds in soil inoculated with teliospores	55.0	65.0
4. Mixing seedlings on moistened cotton wool with dry teliospores	16.2	17.5
5. Mixing seedlings on PDA with dry teliospores	25.0	5.0
6. Uninoculated plants (check)	0	0
Mean	26.45	29.16
CV (%)	9.5	6.7
LSD (0.05)	11.32	12.21

(iv) Inoculation of seedlings on moistened cotton wool technique

Seeds were soaked separately in moistened cotton wool for 48 hours in Petri dishes at room temperature. The germinating seedlings were then transferred to dry sterilized Petri dishes together with the cotton wool from which excess water was drained out. The germinating seedlings were mixed with dry teliospores of *S. cruentum* or *S. reilianum* in the ratio of 10 g of seedling to 1 g of spore. The Petri dishes were incubated at 28±2^{o}C for 5 days. On the sixth day, seedlings were transferred with the adhering cotton wool to the farm and transplanted in the evening (between 1600hrs and 1900hrs) on 10 July, 2010 with seedlings being carefully covered with small quantity of soil. The plants were allowed to reach physiological maturity under rain fed conditions. A check consisted of plots grown from inoculated seedlings (Kutama *et al*., 2011a; Kutama, 2012).

(V) Inoculation of seedlings on PDA technique

Seeds were allowed to imbibe water for 48 hours. Germinating seedlings of each variety were then transferred separately to a PDA-plate. About 0.1 g teliospore powder of *S. reilianum* or *S. cruentum* was sprinkled on the germinating seedlings to establish infection and later incubated at 28±2^{o}C for seven days. Seedlings were carefully transferred and planted on the farm together with the remaining PDA agar. An appropriate check was provided by germinating seedling of each variety on PDA without sprinkling any teliospore and transferring same to the field together with the PDA (Kutama *et al*., 2011a; Kutama, 2012).

RESULTS AND DISCUSSION

Five inoculation techniques (including three conventional and two newly developed techniques) and a check were evaluated in two sorghum genotypes; SSV2006002 and SSV2008089 and for both head and loose smuts. The effect of inoculation techniques on the incidence of head smut in the two sorghum genotypes is shown in Table 1. In this experiment, four out of the five techniques tested were found effective in causing the disease in the two sorghum genotypes with significant difference ($P<0.01$). These inoculation methods were injection, soil, PDA and cotton wool inoculation techniques; in order of decreasing percentage incidence of infection. Stem injection method produced the highest percentage disease incidence in both sorghum genotypes with marginal difference between the genotypes. This is followed by soil inoculation technique. In this trial, seed inoculation technique did not produce any disease in both sorghum genotypes, similar with the control, hence it was found to be ineffective inoculation technique for head smut evaluation.

However, for loose smuts using the same sorghum genotypes and inoculation techniques, all the five techniques were found effective but with significant difference ($P\leq0.01$) among treatments and sorghum genotypes. In this experiment, stem injection method still produced the best result on percentage disease incidence followed by seed and soil and the least was cotton wool inoculation techniques which gave 16.2 % and 7.5 % incidence in the sorghum genotypes SSV2006002 and SSV2008089, respectively (Table 2). Therefore, injection method consistently produced the best results in the two sorghum genotypes and for the two smuts.

Table II. Effect of inoculation technique on the incidence of infection of loose smut in two sorghum genotypes

Inoculation technique	Sorghum loose smut incidence (%) in two genotypes:	
	SSV2006002	SSV2008089
1. Mixing dry teliospores with seeds before sowing	48.8	52.6
2. injecting teliospores into stem	60.4	77.1
3. Sowing seeds in soil inoculated with teliospores	45.0	46.2
4. Mixing seedlings on moistened cotton wool with dry teliospores	16.2	7.5
5. Mixing seedlings on PDA with dry teliospores	32.5	15.0
6. Uninoculated plants (check)	0	0
Mean	33.82	33.1
CV (%)	3.5	4.6
LSD (0.05)	13.32	12.21

Table III. Effect of inoculation technique on the severity of head smut in two sorghum genotypes

Inoculation technique	Sorghum head smut severity in two genotypes:	
	SSV2006002	SSV2008089
1.Mixing dry teliospores with seeds before sowing	0.0	0.0
2. Injecting teliospores into stem	4.0	5.0
3.Sowing seeds in soil inoculated with teliospores	4.0	5.0
4.Mixing seedlings on moistened cotton wool with dry teliospores	3.0	2.0
5.Mixing seedlings on PDA with dry teliospores	3.8	3.1
6. Uninoculated plants (check)	0.0	0.0
Mean	2.46	2.55
CV (%)	1.09	1.09
LSD (0.05)	1.21	1.40

Effects on severity of head and loose smuts

Table 3 shows the mean severity rating of head smut as produced by different inoculation methods. Similar to the incidence earlier mentioned, the mean severity rating (using 1-5 scale) in head smut was higher in stem injection and soil inoculation techniques followed by cotton wool and inoculation of seedlings on PDA in that order of decreasing severity.

On the other hand, the mean severity rating of loose smuts in the two sorghum genotypes due to different inoculation techniques is shown in Table 4. The results on the severity class (also rated on scale of 1-5) indicate that loose smut was not as severe as head smut. The highest was 5.0 obtained in stem injection method for SSV2006002 sorghum genotype and the least 4.0 found in SSV2008089 for cotton inoculation technique resulting into significant difference (P=0.01) between the genotype. Of the five inoculation techniques tested in this experiment, four of them produced results with significant difference. Incidences of both head and loose smuts were highest when injection technique was used to inoculate the two sorghum genotypes. It has been demonstrated that in order to obtain and /or assess smut reaction, researchers typically use different inoculation methods or assays (Olweny *et al*., 2008). Artificial inoculation can be achieved by various inoculation techniques (Ilyas *et al.,* 1992). Selveraj (1980) and Kutama *et al*. (2011a) reported six different inoculation methods for head smut and two for grain and loose smuts. Frowd (1980) described five techniques. In the present study, hypodermic injection method proved to be the best for the inoculation of the two

Table IV. Effect of Inoculation technique on the severity of loose smut in two sorghum genotypes

Inoculation technique	Sorghum loose smut severity in two genotypes:	
	SSV2006002	**SSV2008089**
1.Mixing dry teliospores with seeds before sowing	3.2	5.0
2. Injecting teliospores into stem	3.4	5.0
3.Sowing seeds in soil inoculated with teliospores	0.0	0.0
4.Mixing seedlings on moistened cotton wool with dry teliospores	3.5	4.0
5.Mixing seedlings on PDA with dry teliospores	3.0	2.5
6.Uninoculated plants (check)	0.0	0.0
Mean	2.68	3.25
CV (%)	2.3	1.98
LSD (0.05)	1.56	1.95

sorghum genotypes. In the past, King (1969) employed this technique for screening sorghum collections. He showed that Nigerian sorghum types inoculated by this method showed that up to 71% to 90 % were infected whereas the exotic types were not infected. Wilson and Frederiksen (1970) also injected compatible monosporidial lines and obtained significant infection. Inoculation using injection method was developed by Poehlman (1945) and he found it superior to partial vacuum and other methods. Injection inoculation technique produced the best results probably because by injecting the germinating teliospores, so many mechanisms and or obstacles are overcome in the plant and or in the environment. For example, resistance that only confers disease tolerance is usually eliminated when germinating teliospores are injected into the meristemtic tissue of the growing host plant especially at young seedling stage when the plants do not develop high resistance (Miller, 1978; Sundaram, 1980; Osorion and Frederiksen, 1998). Keay *et al*. (1969) showed that 64 % of the 3-4 weeks old compared with 7-8 weeks old sorghum seedlings become infected when plants were inoculated with hypodermic syringe for loose smut, indicating that sorghum is more susceptible to smut at seedling stage. In this study, injection of germinating spores was done twice, namely at, 3WAS and 40DAS in order to avoid escape. The result of this experiment agrees with the report of Waller (1970) that injection inoculation may induce greater smut infection than other inoculation techniques, and cultivars can respond differently to the two methods of inoculations.

Other techniques that were found to be ineffective for head smut includes; soil, PDA, and cotton wool while for loose smut, soil, and seed inoculations, were effective but PDA and cotton wool were ineffective. Head smut is essentially soil-borne while loose smut is externally seed-borne (Tarr, 1962; Singh, 1998; IPM, 2008). Soil inoculation therefore would obviously yield better results in susceptible varieties for loose smut than for head smut under suitable environmental conditions. However, in loose smut, both seed and soil inoculation techniques produced higher percentage disease incidence with little or marginal differences between the two. The disease was contracted in soil probably because in this experiment, soil and seeds were both mixed with teliospores prior to sowing to obtain maximum infection. Selveraj (1980) recommended seed application technique for the screening covered smut and loose smut since they are similar.

In this trial, two new inoculation techniques were evaluated alongside three old techniques. These were; inoculation of seedlings on PDA, and moistened cotton wool. In both head and loose smuts, the two methods produced some diseased plants although incidences of infection were significantly lower than in two or three older methods. In developing these two techniques, it was postulated that bringing germinating sorghum seedlings in contact with teliospores in a medium conducive for the growth of both, would facilitate and or promote rapid germination of teliospores and the subsequent infection of the young seedlings. However, this was achieved but probably slowly and that was why the percentage incidence was generally low in both cases and in the two sorghum genotypes. Germination of teliospores of *S. relianum* in the soil or in the presence of sorghum seedlings has been reported by Al-Sohaily (1963) and Wilson (1969). Both authors have indicated a very low frequency of germination. The relevance of inoculation techniques relies in the urgent need to explore resistant sorghum varieties among the sorghum germplasm within the country (Kutama *et al*., 2011).

CONCLUSION

Based on the findings of the present research, it is evident that all the inoculation techniques/methods could be employed in the screening of sorghum genotypes against

the two diseases. However, stem injection would produced the best results for both diseases, even though tedious.

REFERENCES

Al-Sohaily I, Mankin ACG, Semeniuk G (1963). Physiologic specialization of *Sphacelotheca reiliana* to sorghum and corn. *Phytopathol*., 53:723-726

Claflin LE, Ramundo BA (1996). Evaluation of all disease and insect sorghum germplas for susceptibility to covered kernel smut. *Phytopathol*., 86: S63 Abstr.

Dogget O (1988). *Sorghum*. 2nd edition. Longman group, U.K Ltd. Pp. 360-365

FAO (1992). Food and Agricultural Organization Statistics. Production year book. Vol.46. Rome: FAO

FAO (2012). Food and Agricultural Organization Statistics. Available @www.fao.org//faostat

Fisher KS, Wilson GL (1975). Studies of grain production in sorghum bicolor L. Moench:III. *Australian J. Agric. Res.,* 26:31-41

Frederiksen RA (1977). Head smuts of corn and sorghum. *Proceedings, 32nd Annual corn and Sorghum Res. Conference* 32:89-105

Frowd JA (1980). A world review of sorghum smuts. In: *Proceedings of the International Workshop on Sorghum Diseases, a World Review held at Hyderabad, India, 11*-15, December, 1978

Gwary DM, Obida A, Gwary SD (2007). Management of Sorghum Smuts and Anthracnose using Cultivar Selection and Seed Dressing Fungicide in Maiduguri, Nigeria. *Inter. J. Agric. and Biol., 9(2):326-328*

Gwary DM, Asala SW (2006). Cost Benefit of Fungicidal Control of Anthracnose in Northern Nigeria. *Inter. J. Agric. and Biol*., 8:306-308

Ilyas MB, Ahmad MI, Bajwa MA (1992). Evaluation of Inoculation Methods and Screening of Wheat Germplasm against Loose Smut Disease. *Pak. J. Agric. Res.,* 27(3):252-256

IPM (2008). Integrated pest management. *Reports on plant disease* (RPD) No.408. Sorghum smuts Pp. 1-6

Komolafe MF, Adegbola AA, Are AL, Ashaye TJ (1985). *Agric. Science for West African Schools and Colleges*. 2nd edition. University Press Limited, Ibadan, pp. 20-120

Keay MA, Dransfield M, McDonald D, King SB, Fowler AM, Quanash SK, Carter JBH (1969). Plant pathology section. *Report* of *the Institute of Agric. Res., Samaru.* 1967-68. 52-61

King SB (1969). *Reports on the Advisory Board on the Institute* (IAR-Samaru) work in 1969. Crops and animals pp.14-15

Kutama AS (2012). Studies on the epidemiology and control of Sorghum Head and Loose smuts in the Sudan savanna region of Nigeria. *Ph.D Thesis* (unpublished), Department of Plant Science, Bayero university, Kano, Nigeria. Pp. 165-180

Kutama AS, Mani AM, Aisha WA (2012). Investigating the nature of seed-borne infection of loose smut induced by *Sporisorium cruentum* (Kuhn) Potter in partially infected sorghum seeds in northern Nigeria, *Savannah J. Agric.,* 7 (2): 1-6

Kutama AS, Aliyu BS, Emechebe AM (2011b). Field screening of sorghum genotypes for resistance to head smut in the Sudan savanna agro-ecological zone of Nigeria. In: *Proceedings of the International Conference on science and Technology held @ Porto Novo, Republic of Benin* 1(7):90-98

Kutama AS, Aliyu BS, Emechebe AM (2011c). Screening of sorghum genotypes for resistance to loose smut in Nigeria. *Bayero J. Pure and Applied Sci.* 4(2):199-203

Kutama AS, Auyo MI, Umar S, Umar ML (2013). Reduction in growth and yield parameters of sorghum genotypes screened for loose smuts in Nigerian Sudan Savanna. *World J. Agric. Res.*1(5):185-192

Kutama AS, Emechebe AM, Aliyu BS (2011a). Field evaluation of some inoculation techniques on the incidence and severity of sorghum head smut (*Sporisorium reilianum*) in Nigerian Sudan savanna. *Biol. and Environ. Sci. J. for the Tropics,* 8(3):292-296

Kutama AS, Emechebe AM, Aliyu BS (2011d). Evaluating the efficacy of seed treatment fungicides in the control of sorghum head smut caused by *Sporisorium reilianum*, in the Sudan savanna region of Nigeria. *J. Phytopathol. and Plant Health* 1:93-98

Marley PS, Gupta SC, Aba DA (2002). Assessment of sorghum genotypes for resistance to foliar Anthracnose (*Colletotrichum graminicola*) under field conditions. *Samaru J. Agric. Res*., 18:17-24

Miller DF (1978). Composition of Cereal grains and Forages. National Academy of Sciences, National Research Council, Washington, DC. Publ. 585

Olweny CO, Ngugi K, Nziok H, Githin SM (2008). Evaluation of Smut Inoculation Techniques in Sugarcane Seedlings. In: *Proceedings of the Conference of South African Sugarcane Technol. Assoc.* 2008, 81:4787-481

Poehlman JM (1945). A simple method for inoculating barley with loose smut. *Phytopathol.* 35:460-465

Selveraj JC (1980). Smut Research and Control in Nigeria in: *Proceedings of the International workshop on sorghum diseases, a world review held at Hyderabad, India,*11-15, December,1978

Singh RS (1998). *Plant Diseases*. Seventh edition. Oxford and IBH Publishing Co.PVT.LTD. pp.335-34

Sundaram NV (1980). Importance of Sorghum Smuts in African Countries. In: *Proceedings of the International Workshop on Sorghum Diseases, a World Review held at Hyderabad, India*,11-15, December,1978

Tarr SAJ (1962). *Diseases of Sorghum, Sudan grass and Browncorn.* Commonwealth Mycological Institute, Kew, Survey Pp. 171-247

Waller JM (1970). Sugarcane Smut (*Ustilago scitaminea*) in Kenya II: Infection and Resistance. *Trans. Br. Mycol. Soc*. 54:405-414