Inheritance of Resistance to Ergot Disease in a Diallel Cross of Pearl Millet (*Pennisetum glaucum* (L.) R. Br.)

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Abstract

A field study was conducted to incorporate resistance to ergot and determine its inheritance in four pearl millet hybrids (SOSAT C88, Ex-Borno, LCIC 7902 and PEO 5948) from three resistant local pearl millet landraces (Geron Tsuntsu, Zango and Dandigali). Crosses of the seven pearl millet lines were carried out in all possible combinations but without reciprocals using diallel mating design to generate 21 F_1 populations during the 2011 dry season to March 2012 under irrigation system at the University of Maiduguri Teaching and Research Farm, Nigeria. The seven parental lines and 21 F_1 hybrids were evaluated for ergot disease, days to 50% flowering and grain yield in a randomized complete block design (RCBD) with three replications during the 2012 rainy season in Gombe, Nigeria. Results showed that each of the seven parent lines attained 50% flowering at statistically similar days with their respective crosses except for SOSAT C88 (SOSAT C88 vs Dandigali and LCIC 9702), Zango (Zango vs SOSAT C88, LCIC 9702 and Dandigali) and PEO 5948 x LCIC 9702. This synchrony provided a uniform condition and duration for evaluating the resistance to ergot infection in the crosses vis-à-vis the parent cultivars. F_1 between either SOSAT C88, Ex-Borno or LCIC 7902 and each of landrace cultivars showed positive heterosis for ergot disease. Zango x Dandigali and Geron Tsuntsu x PEO 5948 showed the lowest incidence (15.00%, 16.67% respectively) while SOSAT C88 and SOSAT C88 x Ex-Borno gave the highest (50.00%, 45.00% respectively). Lowest ergot severity was recorded from F_1 between Dandigali and either Ex-Borno (6.17%) or Geron Tsuntsu (6.33%) although not significantly (p=0.05) different from Geron Tsuntsu x PEO 5948 (08.08%) while SOSAT C88 and LCIC 9702 had the highest severity (31.61%, 33.25% respectively). SOSAT C88 x Zango gave significantly the highest grain yield (391.81 kg/ha) while SOSAT C88 recorded the lowest grain yield (101.11 kg/ha). The results of the study showed that the local landraces of pearl millet used in the study have genetic potential to manage ergot disease in hybrids of pearl millet due to their ability to confer resistance and desirable heterosis for disease and yield in their F_1. Multi-locational trials need to be conducted on the crosses that showed resistance to ergot to confirm the stability and durability of their resistance.

Keywords: Claviceps fusiformis, diallel mating design, incidence, pearl millet, severity.

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a staple food for millions of poor people living in the semi-arid tropical regions of Africa and Asia (Govindaraj *et al.,* 2019). Cultivation of the crop predominates drier parts of all the geographical zones of Africa and the Indian Subcontinent of South Asia (Williams and Andrews, 1983, Thakur *et al.,* 2011, Yadav and Rai, 2013). More than half of the world's millet production lies in Asia and Africa on 26 million hectares with about 70% of it in West Africa (Yadav and Rai, 2013). The crop is a very important cereal crop in the Savannah and Sahel areas of Nigeria where it is second to sorghum in importance (Ajayi *et al.,* 1998; Abraham *et al.,* 2019a). Of the 32 million tons of global millet grain yield produced, about 90% is utilized in developing countries (DAFFRSA, 2011; FAO, 2018). It is estimated that a total of 20 million tons is consumed as food in a form of porridge, couscous, snacks, etc., in developing countries of Africa and Asia, while the rest is being used for animal feed and other industrial uses such as preparation of local alcoholic beverages (Aliyu *et al.,* 2011; FAO, 2018). Despite the importance of the crop in Nigeria and elsewhere, the prevalence of abiotic and biotic factors limits its profitable production (Rai *et al.,* 1999; Kanfany *et al.,* 2018; Abraham *et al.,* 2019b). Ergot caused by *Claviceps fusiformis*, is considered one of the economically important biotic constraints to pearl millet production in Asia, Africa and North America (Thakur and Rai, 2002; CABI/EPPO, 2019). In Nigeria, it is one of the major diseases of pearl millet and is endemic in North-Western and North-Eastern parts of the country (reviewed by Abraham *et al.,* 2016). The disease is more severe in genetically uniform single-cross F_1 hybrids, having a significant impact on yields, with up to 70% loss of both grain yield and quality under favorable weather condition (Natarajan *et al.,* 1974; Thakur *et al.,* 2011, Klotz and Smith, 2015). In addition to reducing grain yield ergot adversely contaminates grain with neurotoxic, alkaloid-containing sclerotia thus creating a health hazard for consumers (Werder and Manzo 1992; Haarmann *et al.,* 2009). The uses of fungicides such as ziram and cabendazim have been reported to be effective against ergot in pearl millet (Kumar and Thakur, 1996; Alderman, 2006). However, continued application of these chemicals leads to emergence of resistant pathogenic races, increased production cost and negative effect on the environment and human health (Sasode *et al.,* 2018). Cultural practices such as the use of ergot-free seeds, removal of sclerotia by floating millet seeds in 10% salt solution, crop rotation, intercropping, elimination of plant debris, deep ploughing and adjustment of sowing date, etc., have been reported to reduce infection by *C. fusiformis* in pearl millet (Thakur, 1983; Randhawa *et al.,* 1992; Kumar and Thakur, 1995; Thakur, 1998). Pearl millet is grown largely by resource–poor farmers under subsistence agriculture in the Semi-arid tropics (Wilson, 2000) and ergot is an airborne and soil-borne disease (Tooley *et al.,* 2001; Anitha *et al.,* 2005; Zida *et al.,* 2008). Therefore, the use of host-plant resistance remains the most effective, economical and environmentally sustainable method of managing ergot in pearl millet (Andrews *et al.,* 1985; Hash *et al.,* 1999; Thakur and Rai, 2002; Thakur *et al.,* 2011). Local cultivars of pearl millet have been reported to be generally resistant to ergot while F_1 hybrid lines are generally susceptible (Thakur *et al.,* 1985; Meidaner and Geiger, 2015). It is important that pearl millet improvement programmes utilize locally resistant cultivars as the basis for crop improvement, since exotic, so-called elite, improved millets mostly developed in India are generally, in Africa for example, highly susceptible to local strains of pathogens (Williams and Andrews, 1983; Thakur *et al.,* 1985; Thakur and Rai, 2002). The objectives of the study were to incorporate resistance to ergot and determine its inheritance in four pearl millet hybrids (SOSAT C88, Ex-Borno, LCIC 9702 and PEO 5948) using three ergot resistant pearl millet landraces (*Zango, Geron Tsuntsu* and *Dandigali*) in Gombe.

Materials and Methods

Seven (7) pearl millet lines which include three ergot resistant landraces (*Geron Tsuntsu, Zango* and *Dandigali*) and four hybrids (SOSAT C88, Ex-Borno, LCIC 9702 and PEO 5984) were selected for the study based on their geographical diversity, variability in the yield, agronomic attributes and reaction to ergot disease (Table 1). The four hybrid cultivars were obtained from Lake Chad Research Institute Maiduguri, Borno State while the three land race cultivars were obtained from Kembu in Akko Local Government Area (LGA) of Gombe State. Crosses of the seven pearl millet lines were carried out in all possible directions (diallel mating design) without reciprocals during the 2011 to 2012 dry season under irrigation system at the University of Maiduguri Teaching and Research Farm. The seven cultivars were designated as both males and females parent whose seeds were hand sown as a pinch at a spacing of 75 cm x 50 cm in a 2 rows, 5 meters long plot and later thinned to two plants per stand after 3 weeks of sowing. A mixed fertilizer (N P K 20:10:10) was applied at the elemental equivalent of N at 60 kg/ha, 30 kg/ha of P_2O_4 and

Table 1: Description of seven experimental materials LCRI= Lake Chad Research Institute

Cultivar	Source	Days to 50% Flowering	Description
Geron Tsuntsu	Kembu	65	Long hairy panicle, dark grey and medium sized seeds, late maturing, low yielding, resistant to Downy mildew, Smut and Ergot and adapted to the Sudan and Sahel regions.
Zango	Kembu	75	Very long, semi compact has paniclet at the base of its panicle, late maturing, low yielding, resistant to Downy mildew, Smut and Ergot and adapted to the Sudan and Sahel regions.
Dandigali	Kembu	70	Small panicles (candle in shape) semi-compact medium sized seeds, late maturing, low yielding, resistant to Downy mildew, Smut and Ergot and adapted to the Sudan and Sahel regions.
SOSAT C88	LCRI	53	Long, cylindrical and compact panicle, large seeds, early maturing, high yielding, resistant to Downy mildew and Smut, susceptible to Ergot and adapted to the Sudan and Sahel regions
Ex-Borno	LCRI	60	Medium sized seed, medium maturing, high yielding, susceptible to Downy mildew, Smut and Ergot and adapted to the Sudan and Sahel regions.
LCIC 9702	LCRI	57	Long compacted and candle-like shaped panicles, early maturing, large seeds, high yielding, susceptible to Downy mildew and Smut, moderate resistance to Ergot and adapted to the Sudan and Sahel regions.
PEO 5984	LCRI	60	Panicles are average in length with thick girth, large seeds medium maturing, high yielding, susceptible to Downy mildew, Smut and Ergot and adapted to the Sudan and Sahel regions.

LCRI= Lake Chad Research Institute
Source: Izge, 2006; Gaya *et al.* 2012.

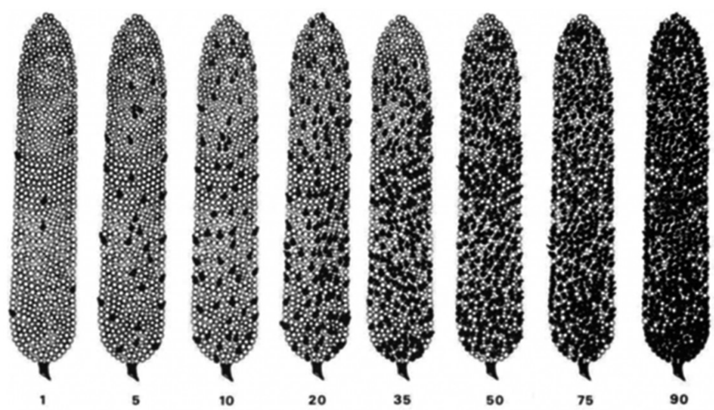

Figure 1: Ergot severity (%) rating scale (Thakur and King 1988a)

K_2O respectively in splits at 2 and 6 weeks after sowing. Weeds were managed as described by Onwueme and Sinha, (1991). The seven parents were stagger planted to synchronize flowering to obtain sufficient seeds. Matured seeds of 7 parent lines and 21 F_1 hybrids were harvested and further sun dried at 10 % moisture content.

The 21 F_1 progenies were evaluated along with their seven parents during the 2012 rainy season at Dukke village in Akko LGA of Gombe State (10°8'N and 11°20'S), Nigeria. The agro-ecological zone of Dukke is characterized by a sandy loam soil, an average rainfall of about 886.5mm per annum and annual average temperature of 20-35°C. The experimental site has been under millet cultivation for over 20 years which most likely means well built up disease inoculum in the site. Natural epiphytotic in the fields was therefore relied upon as the source of primary and secondary inoculum. The experiment was laid out in randomized complete block design (RCBD) with each plot size having 4 rows and 5 m long each in to which 21 F_1 progenies and their seven parent lines were randomly assigned and replicated three times. All the agronomic practices carried out during the crossing of the seven parent lines under irrigation were also applied during the evaluation of the crosses and their parent lines under rain fed conditions. Data were collected as described by Izge *et al.* (2007) on twenty plants from net rows of each plot and their means were computed for the following parameters: days to 50% flowering was determined from the day of sowing to when 50% of the plants reached anthesis (start booting); ergot incidence (%) was computed as number of diseased plants expressed as percentage of the total number of plants assessed. For ergot severity (%), each panicle was scored for severity using the standard ergot disease rating scale (0 to 100% scale) to estimate the percentage of florets infected as described by Thakur and King, 1988a (Fig. 1); then means of each treatment computed. Harvested dried panicles were threshed and grains obtained per plot weighed and used to determine grain yield in kg ha^{-1}.

Data collected were subjected to analysis of variance and treatment means were separated based on Duncan Multiple Range Test at 5% level of probability using Analyzed-it® v 2.10 (Analyzed-it, 2007).

Results and Discussion

The result (Table 2) showed that all of the seven parental cultivars attained 50 % flowering at later days than days reported by Izge, (2006) and Gaya *et al.* (2012). Environmental or physiological factors could be the reason for this. Each of the seven parent lines attained 50% flowering at statistically similar days with their respective crosses except for SOSAT C88 (SOSAT C88 vs *Dandigali* and LCIC 9702), *Zango* (*Zango* vs SOSAT C88, LCIC 9702 and *Dandigali*) and PEO 5948 x LCIC 9702. This indicated that the F_1 had a neutral flowering heterosis which contradicts the negative (and desirable) heterotic effect reported by Izge (2006). The result (Table 2) however, provided a uniform condition for both the parent lines and their crosses to be exposed to the pathogen inoculum. As expected, all the three landrace cultivars (*Geron Tsuntsu, Zango* and *Dandigali*) showed significantly lower ergot incidence and severity compared to three hybrid parents (SOSAT C88, Ex–Borno and LCIC 9702) as seen in Table 2. Works of several authors (Williams and Andrews, 1983; Thakur and Rai, 2002; Miedaner and Geiger, 2015) reported that landraces in West Africa undergo random cross pollination where considerable genetic variability occurs among the plants which enable the crop to reduce the effect of specific stress factors including plant pathogens. The evolution of pearl millet-*Claviceps fusiformis* system has been in a state of epidemiological equilibrium within the local landraces of pearl millet composed of heterogeneous plant populations (Thakur *et al.,* 1985). PEO 5948 had significantly the lowest ergot incidence and severity than any of the hybrid parents (Table 2). This further confirmed its moderate genetic resistance (Gaya *et al.,* 2012). Crosses (F_1 generations) of either SOSAT C88, Ex-Borno, PEO 5948 or LCIC 9702 with each of the landrace cultivar had significantly suppressed incidence and severity of ergot than their hybrid parents except for PEO 5948 x *Zango*

Table 2: Days to 50% flowering, ergot incidence (%), ergot severity (%) and yield (Kg/Ha) of parent lines of pearl millet and their crosses during the 2012 cropping season in Gombe

Parent /Crosses	Days to 50% Flowering	Incidence (%)	Severity (%)	Yield (Kg/Ha)
SOSAT C88	78.33[abc]	50.00[a]	31.61[a]	101.11[u]
Geron *Tsuntsu*	73.67[abcdef]	26.67[ghi]	09.891[m]	195.37[n]
Ex- Borno	74.00[abcdef]	43.33[bc]	25.28[bc]	327.49[f]
Zango	79.33[a]	25.00[ghi]	15.39[hij]	110.91[t]
LCIC 9702	72.33[cdef]	43.33[bc]	33.25[a]	183.90[o]
Dandigali	75.00[abcdef]	21.67[ij]	20.67[defg]	155.54[q]
PEO 5942	79.00[ab]	23.33[hi]	11.28[klm]	151.33[q]
SOSAT C88 × Geron *Tsuntsu*	73.33[abcdef]	35.00[de]	17.88[gh]	202.23[m]
SOSAT C88 × Ex- Borno	76.33[abcde]	45.00[ab]	23.55[bcd]	246.42[i]
SOSAT C88 × *Zango*	72.33[cdef]	33.33[def]	24.08[bc]	391.81[a]
SOSAT C88 × LCIC 9702	71.33[ef]	31.67[efg]	15.88[hi]	327.49[f]
SOSAT C88 × *Dandigali*	70.00[f]	41.67[bc]	19.17[fg]	333.21[e]
SOSAT C88 × PEO 5942	73.00[abcdef]	31.67[efg]	22.36[cdef]	354.63[c]
Geron *Tsuntsu* × Ex- Borno	72.00[def]	23.33[hi]	15.17[hij]	342.25[d]
Geron *Tsuntsu* × *Zango*	78.00[abcd]	31.67[efg]	22.86[bcde]	142.49[r]
Geron *Tsuntsu* × LCIC 9702	72.00[def]	30.00[efg]	23.84[bcd]	327.85[f]
Geron *Tsuntsu* × *Dandigali*	74.00[abcdef]	23.33[hi]	06.33[n]	208.90[l]
Geron *Tsuntsu* × PEO 5942	71.00[ef]	16.67[jk]	08.08[mn]	300.02[g]
Ex-Borno × *Zango*	77.00[abcde]	21.67[ij]	13.61[ijk]	225.91[j]
Ex-Borno × LCIC 9702	75.33[abcdef]	28.33[fgh]	26.00[b]	262.30[n]
Ex-Borno × *Dandigali*	77.00[abcde]	23.33[hi]	06.17[n]	181.84[o]
Ex-Borno × PEO 5942	79.00[ab]	30.00[efg]	22.39[cdef]	133.34[s]
Zango × LCIC 9702	73.00[bcdef]	30.00[efg]	12.50[jkl]	300.02[g]
Zango × *Dandigali*	71.67[ef]	15.00[k]	13.28[ijk]	165.12[p]
Zango × PEO 5942	74.67[abcdef]	28.33[fgh]	13.17[ijk]	379.05[b]
LCIC 9702 × *Dandigali*	74.00[abcdef]	26.67[ghi]	20.11[efg]	215.55[k]
LCIC 9702 × PEO 5942	72.67[cdef]	38.33[cd]	23.44[bcd]	165.49[p]
Dandigali × PEO 5942	79.00[ab]	38.33[cd]	22.55[cde]	162.78[p]
S.E (±)	3.017	3.254	1.627	2.614

Means within column followed by similar letter(s) are not significantly different at p=0.05 using Duncan Multiple Range Test and PEO 5948 x Dandigali (Table 2). This implied that these F_1 generations had positive heterotic effect, which is desirable for disease incidence and severity (Ouendeba *et al.,* 1993). It could also indicate evidence of inheritance of resistance controlled by several genes in the F_1 generations (Thakur *et al.,* 1983). PEO 5948 parent showed higher resistance to the disease than its crosses with either *Zango* or *Dandigali*. This could imply incompatibility (negative general combining ability (GCA) between the parents or recesiveness of the resistant genes in F_1 generations (Thakur *et al.,* 1985; Thakur *et al.,* 2011). The diallel cross between hybrid parents showed that SOSAT C88, LCIC 7902 and Ex-Borno x LCIC 7902 crosses significantly suppressed ergot incidence and severity than their parents except for severity in Ex-Borno. This also gave a desirable negative heterosis but contradicts the research findings of Thakur *et al.* (1985) and Mbwaga and Mdolwa (1995) who reported that crosses from ergot susceptible hybrids become more susceptible than their parents. Cross between PEO 5948 and either SOSAT C88, LCIC 7902 or Ex-Borno had negative heterosis on ergot resistance except for PEO 5948. It could be interpreted here that PEO 5948 had a positive general combining ability with the three other hybrid parents and transferred resistant genes in them. The higher susceptibility of SOSAT C88 x Ex-Borno cross than both hybrid parents could be attributed to presence of susceptible cytoplasmic germplasm (Rai and Thakur 1995; Thakur and Rai, 2002). A cross between *Zango* and *Dandigali* showed significantly lower ergot incidence than both parents and also low severity than *Dandigali* (Table 2). Lower ergot severity was recorded in Geron *Tsuntsu* x *Dandigali* cross than both parents (Table 2). Williams and Andrews (1983) and Thakur *et al.* (1985), reported that a cross between moderate or less ergot susceptible millet usually shows higher resistance than its parents. However, Geron *Tsuntsu* x *Zango* cross did not suppress the disease better than its parents. This could be due to general incompatibility (negative GCA) of the parents. Of all the entries, crosses between *Dandigali* and either Ex-Borno or Geron *Tsuntsu* had the lowest ergot severity. *Zango* x *Dandigali* and Geron Tsuntsu x PEO 5948 showed the lowest ergot incidence while SOSAT C88 had the highest incidence and severity (Table 2). Among the crosses of Ex-Borno with each of the landrace cultivar, only Ex-Borno x Geron *Tsuntsu* had grain yield significantly higher than its hybrid parent (Ex-Borno). Among all the crosses, SOSAT C88 x *Zango* had the highest grain yield while Ex-Borno x PEO 5948 gave the lowest (Table 2). From all the entries, SOSAT C88 x *Zango* had the highest grain yield while SOSAT C88 recorded the lowest. Natarajan *et al.* (1974) and Kumar *et al.* (1997) observed that significant grain yield losses were incurred on susceptible pearl millet hybrids due to ergot attack. F_1 between either SOSAT C88, LCIC 9702 or PEO 5948 and each of the landrace cultivars gave significantly higher grain yields than their respective hybrid parents (Table 2). Higher ergot incidence and severity of SOSAT C88 and LCIC 7902 could have likely affected their yields (Mangat *et al.,* 1996).

Conclusion

From the findings of this study, it can be concluded that the local landraces of pearl millet (Geron *Tsuntsu*, *Zango* and *Dandigali*) used in the study showed great genetic potential for the management of ergot disease in hybrid of pearl millet due to their ability to confer resistance and desirable heterosis for disease and yield in their F_1. Local landraces of pearl millet should therefore be explored to manage other economic diseases of pearl millet through breeding programme in Nigeria. Multi-locational trial needs to be conducted on the crosses that showed resistance to ergot to confirm the stability and durability of their resistance.

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