Field Crops Research 118 (2010) 169–176

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Heritability of, and genotypic correlations between, aflatoxin traits and physiological traits for drought tolerance under end of season drought in peanut (*Arachis hypogaea* L.)

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ARTICLE INFO

Article history:
Received 24 December 2009
Received in revised form 18 May 2010
Accepted 18 May 2010

Keywords:
Arachis hypogaea L.
Aflatoxin
A. flavus
SLA
SCMR
Drought resistance

ABSTRACT

More rapid progress in breeding peanut for reduced aflatoxin contamination should be achievable with a better understanding of the inheritance of, aflatoxin trait and physiological traits that are associated with reduced contamination. The objectives of this study were to estimate the heritability of aflatoxin traits and genotypic (r_G) and phenotypic (r_P) correlations between drought resistance traits and aflatoxin traits in peanut. One hundred-forty peanut lines in the $F_{4:6}$ and $F_{4:7}$ generations were generated from four crosses, and tested under well-watered and terminal drought conditions. Field experiments were conducted under the dry seasons 2006/2007 and 2007/2008. Data were recorded for biomass (BIO), pod yield (PY), drought tolerance traits [harvest index (HI), drought tolerance index (DTI) of BIO and PY, specific leaf area (SLA), and SPAD chlorophyll meter reading (SCMR)], and aflatoxin traits [seed infection and aflatoxin contamination]. Heritabilities of *A. flavus* infection and aflatoxin contamination in this study were low to moderate. The heritabilities for seed infection and aflatoxin contamination ranged from 0.48 to 0.58 and 0.24 to 0.68, respectively. Significant correlations between aflatoxin traits and DTI (PY), DTI (BIO), HI, biomass and pod yield under terminal drought conditions were found ($r_P = -0.25^{**}$ to 0.32^{**}, $r_G = -0.57^{**}$ to 0.53^{**}). Strong correlations between SLA and SCMR with *A. flavus* infection and aflatoxin contamination were also found. Positive correlations between SLA at 80, 90, and 100 DAP and aflatoxin traits were significant ($r_P = 0.13^{**}$ to 0.46^{**}, $r_G = 0.26^{**}$ to 0.81^{**}). SCMR was negatively correlated with aflatoxin traits ($r_P = -0.10^{**}$ to -0.40^{**}, $r_G = -0.11^{**}$ to -0.66^{**}). These results indicated that physiological-based selection approaches using SLA and SCMR might be effective for improving aflatoxin resistance in peanut.

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1. Introduction

Preharvest aflatoxin contamination (PAC), induced by terminal drought and heat stress, in peanut (*Arachis hypogaea* L.) is an important quality problem with serious health concern worldwide. Aflatoxins, which are toxic secondary metabolites, are well recognized as potent carcinogenic, teratogenic and immunosuppressive substances (Turner et al., 2000; Wild and Hall, 2000; Hall and Wild, 2003) produced when toxigenic strains of the fungi *Aspergillus flavus* Link. ex Fries and *A. parasiticus* Speare grows on peanuts subjected to drought (Blankenship et al., 1984). Hence, a solution for eliminating or reducing PAC is necessary. Late season irrigation to alleviate drought stress of plants is effective in reducing PAC in the field (Dorner et al., 1989). However, cultivars with resistance to PAC are still needed, especially at locations where irrigation is not available.

Reduction of PAC through genetic manipulation has been attempted in breeding programs in many countries. However, identification of aflatoxin resistance traits and incorporation of pertinent traits into peanut has been a challenge for breeders. Genotype × environment (G × E) interactions are the main factor hindering the progress of breeding programs for lower PAC, and consistency and accuracy in field experimentation has been difficult to achieve (Anderson et al., 1995, 1996; Holbrook et al., 1994). Seed colonization can only be used as an initial screen because of the generally poor correlation between fungal growth and aflatoxin production. On the other hand, screening for resistance to PAC is also limited by the expense of directly measuring aflatoxin content. Thus, an indirect measure of PAC resistance in peanut is needed to accelerate progress in breeding programs.

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0378-4290/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.fcr.2010.05.007

PAC may be reduced with improved resistance to drought (Cole et al., 1993; Holbrook et al., 2008, 2009). Recent studies have shown a relationship of increased drought tolerance and reduced aflatoxin production (Arunyanark et al., 2009; Girdthai et al., 2010; Holbrook et al., 2000). However, improvement of drought resistance based on yield is also hindered by high G × E interactions (Jackson et al., 1996; Araus et al., 2002). Drought resistance traits with lower G × E interactions are promising as indirect selection tools for improving resistance to PAC. Nigam and Aruna (2008) suggest that the SPAD chlorophyll meter reading (SCMR) and specific leaf area (SLA), are simple and stable drought resistance traits that are easy to measure in large breeding populations. Arunyanark et al. (2009) found significant relationships between physiological traits for drought resistance such as SLA, root length density (RLD), and chlorophyll density (ChlD), with aflatoxin contamination under long-term drought. Girdthai et al. (2010) also found that SLA, relative water content, ChlD, and drought stress ratings are the best traits to use as indirect selection tools for lower PAC under terminal drought conditions. Thus, physiological traits for drought tolerance may help breeders to reduce aflatoxin contamination in peanut.

Few studies to date have investigated the inheritance of aflatoxin traits in peanut under drought conditions. Arunyanark et al. (2010) found moderate heritabilities for seed infection and aflatoxin contamination. They also found that aflatoxin traits were genetically correlated with drought tolerance traits, especially with HI, SLA and SCMR. However, they did not focus on terminal drought which is the most important period for PAC. The effectiveness of mechanisms of drought resistance is dependent on the timing and duration of drought stress. Drought escape mechanisms play an importance role under terminal drought which differs from long period drought (Subbarao et al., 1995; Clavel et al., 2004). From this perspective, the inheritance of aflatoxin traits under long-term and terminal drought might be different.

To develop proper breeding strategies for incorporating resistance to drought and PAC, a breeder must identify sources of resistance, and determine the genetic control of resistance. Specific research on sources of resistance to aflatoxin in peanut has been conducted, but research on inheritance to elucidate the gene action controlling resistance to drought and PAC and to develop improved screening strategies has been limited. Hence, the objectives of the present study were to estimate the heritability of aflatoxin traits and genotypic and phenotypic correlations between drought resistance traits and PAC in peanut in order to predict indirect responses of PAC thought selection for drought resistance traits.

2. Materials and methods

2.1. Genetics materials and experimental design

Four populations developed by crossing 2 drought resistant genotypes, ICGV 98348 and ICGV 98353, with 2 commercial cultivars, KK 60-3 and Tainan 9, were used to study inheritance of resistance to drought and PAC. Two peanut genotypes [ICGV 98348 and ICGV 98353; medium maturing (110 days to maturity) and medium seeded type] are elite drought-resistant lines obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) having low PAC and SLA with high pod yield and SCMR under drought stress (Girdthai et al., 2010). KK 60-3 [late maturing (120 days to maturity) and large seeded type] selected for high PAC, SCMR and biomass with low SLA, and Tainan 9 [early maturing (100 days to maturity) and medium seeded type] selected for high PAC and SCMR with low SCMR and biomass (Girdthai et al., 2010; Puangbut et al., 2009; Songsri et al., 2009) are released cultivars and widely grown in Thailand. Four F_1 hybrids (ICGV 98348 × KK 60-3, ICGV 98348 × Tainan 9, ICGV 98353 × KK 60-3, and ICGV 98353 × Tainan 9) were obtained from the hybridization. The F_1 seeds were planted and their seeds harvested in bulk for each cross. In F_2 and F_3 generations, one pod was kept from each plant and bulked for each cross. Line separation was carried out in the F_4 generation. A total of 140 lines (35 lines for each cross) were randomly selected and multiplied in the F_5 generation.

Four parental lines and the 140 progenies from 4 crosses were evaluated in the $F_{4:6}$ and $F_{4:7}$ generations (F_4-derived lines in the F_6 and F_7 generations, respectively) under two soil moisture levels [field capacity (FC) and 1/3 available soil water (1/3 AW) at 80 days after planting (DAP) to final harvest] for two years in the dry season 2006/2007 and repeated in the dry season 2007/2008. A split plot design with four replications was used for both years at the Field Crop Research Station, Faculty of Agriculture, Khon Kaen University located in Khon Kaen Province, Thailand (latitude 16°28′N, longitude 102°48′E, 200 m above sea level). Soil type is Yasothon Series (loamy sand, Ocix Paleustults) with 10.2% soil moisture at FC and 3.1% at permanent wilting point. Two soil moisture levels, FC (10.2%) and 1/3 AW (5.5%) in 0–60 cm depth were assigned as main plots, and peanut lines were laid out in subplots. Each entry was planted in five row plots with 3 m length. Spacing was 40 cm between rows and 20 cm between plants within the row.

2.2. Crop management

Soil was prepared by ploughing the field three times. Lime at the rate of 625 kg ha^{-1} was applied at first ploughing. Nitrogen fertilizer as urea at the rate of 31.1 kg N ha^{-1}, phosphorus fertilizer as triple superphosphate at the rate of 24.7 kg P ha^{-1} and potassium fertilizer as potassium chloride at the rate of 31.1 kg K ha^{-1} were incorporated into the soil by broadcasting during soil preparation prior to planting. Seeds were treated with captan [3a,4,7,7a-tetrahydro-2-((trichloromethyl)thio)-1H isoindole-1,3(2H)-dione] at the rate of 5 g kg^{-1} seeds before planting, and seeds of the large seeded genotypes were treated with ethrel (2-chloroethylphospphonic acid) 48% at the rate of 2 ml L^{-1} water to break dormancy. The seeds were over planted and later the seedlings were thinned to obtain one plant per hill at 14 DAP. Weeds were controlled by the application of alachlor [2-cholro-2′,6′-diethyl-N-(methoxymethyl) acetanilide 48% (w/v), emulsifiable concentrate] at the rate of 3 L ha^{-1} at planting and hand weeded during the remainder of the season. Gypsum ($CaSO_4$) at the rate of 312 kg ha^{-1} was applied at 47 DAP. Carbofuran [2,3-dihydro-2,2-dimethylbenzofuran-7-ylmethylcarbamate 3% granular] was applied at the pod setting stage. Pests and diseases were controlled by weekly applications of carbosulfan [2-3-dihydro-2,2-dimethylbenzofuran-7-yl (dibutylaminothio) methylcarbamate 20% (w/v), water soluble concentrate] at the rate of 2.5 L ha^{-1}, methomyl [S-methyl-N-((methylcarbamoyl)oxy) thioacetimidate 40% soluble powder] at the rate of 1.0 kg ha^{-1} and carboxin [5,6-dihydro-2-methyl-1,4-oxathine-3-carboxanilide 75% wettable powder] at the rate of 1.68 kg ha^{-1}.

2.3. Water management

A subsurface drip irrigation system (Super typhoon®; Netafim Irrigation Equipment & Drip Systems, Tel Aviv, Israel) with a distance of 20 cm between emitters was installed with a spacing of 40 cm between drip lines at 10 cm below the soil surface midway between peanut rows to supply water to the crop. Drip lines were fitted with a pressure valve and a water meter to ensure a uniform supply of the required amounts of water. Soil water level was maintained at FC at 0–60 cm depth. This soil depth should reasonably cover the majority of the rooting zone. In stress treatments, water was withheld at 60 DAP for 20 days according to 20 years