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Full Length Research Paper

Study of root traits of chickpea (*Cicer arietinum* L.) under drought stress

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Roots are among the first defence towards drought with other morpho-physiological and biochemical mechanisms employed by plants. To understand precisely the root traits contribution towards yield, parental chickpea genotypes with well known drought response were field evaluated under drought and optimal irrigation in rain-out shelter. A total of ten genotypes planted in 1.2 m PVC lysimeters were subjected to three water stress levels: high moisture stress, medium water stress, and low water stresses. Root traits, such as root length density, total root dry weight, root dry weight and root: shoot ratio, were measured at 40 days after sowing. The roots were washed and scanned using WinRHIZO software. The ANOVA showed that there was significant difference ($P < 0.05$) in traits measured amongst test genotypes which included shoot biomass, root biomass, total root length (RL) and root length density (RLD). The results also showed that there were significant variations ($P < 0.05$) in water regimes and traits decreased with increasing moisture stress from low to high moisture regime. Furthermore, there were variations in root anatomy between the two major chickpea types where majority of the best performing genotypes under low moisture regimes were of the Desi type (e.g. ICC 4958, ICCV 00108, ICCV 92944 and ICCV 92318) as compared to Kabulis which had better and higher response under high moisture regime in this study. These traits could be used for indirect selection for drought tolerance especially in early stages of breeding for drought tolerance which would consequently reduce the cost of multi-location field evaluation in the breeding programs.

Key words: Genotypes, Chickpea *Cicer arietinum* L., drought stress, root traits.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the world's third most important grain-legume crop after beans and pea (Food and Agriculture Organisation, 2012). It is particularly an important crop for the farmers mainly living in sub-Saharan Africa (SSA), and south East Asia (SEA). This is because it is a key component in the diets of resource-poor people who cannot afford to supplement their diets with animal protein (International Crops Research Institute for semi-arid Tropics, 2009). In addition, chickpea is also rich in minerals, vitamins, and dietary

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fibre. Globally, total production is approximately 14.2 million tons from an area of 14.8 million ha and a productivity of 0.96 t ha^{-1} (FAOSTAT, 2014). South East Asia, led by India is leading producers, while in East Africa, Ethiopia, Tanzania, Malawi, and Kenya are leading chickpea producers. Worldwide chickpea is largely grown as a rain fed crop (> 90%) in the arid and semi-arid environments in Asia and Africa (Kumar and Abbo, 2001), where the annual rainfall is received during the preceding rainy season (April-September) and the crop grows and matures on a progressively depleting soil moisture profile (Kashiwagi et al., 2013) and generally experiences terminal drought stress (DS).

In many regions of East Africa, chickpea is usually sown during short rains and under stored soil moisture, with very little rainfall during the cropping season; this leads to constantly receding intensities of water deficit as the crop cycle advances, leading to a severe water deficit at crop maturity, reducing yields significantly. These types of receding soil water conditions impose a ceiling on the cropping duration demanding selection for matching duration varieties for the best adaptability and productivity (Saxena, 1987; Ludlow and Muchow, 1990). As a result, terminal drought is considered as the most serious constraint in chickpea production (Pooran et al., 2008). The loss experienced in chickpea production globally due to terminal drought is estimated to be approximately 50% of the potential production (900 million US dollars). In Kenya, however, chickpea production and area under cultivation has fluctuated over the years and it has declined steadily from 51,772 ha in 2000 to only less than 8000 ha in 2016. Similarly, yield per hectare declined from 4.5 to 2.6 t/ha over the same period (FAOSTAT, 2014). The declining production and area are due to drought, pests and diseases, and limited market outlets since the crop is mostly utilized by the Indian community in Kenya (Kimurto et al., 2005: Kosgei, 2015). Therefore there is need of developing drought tolerant genotypes for production in these areas with best adaptability and productivity. Genetic improvement for better drought adaptation can be along-lasting and less-expensive solution for drought management than the agronomic options. But, due to the numerous mechanisms that plants employ to maintain growth under low water supply, understanding yield maintenance under DS becomes increasingly difficult (Tuberosa and Salvi, 2006). Consequently, a trait-based breeding approach is being increasingly emphasized over grain-yield-based breeding for realizing better stability as grain yields are heavily influenced by high genotype × environment (G × E) inter-actions and exhibit low heritability (h^2) (Ludlow and Muchow, 1990). Also, a trait-based breeding increases the probability of crosses resulting in additive gene action (Reynolds et al., 2007; Wasson et al., 2012). However, knowledge of the type and intensity of DS and the various traits and mechanisms employed by the plant to sustain productivity under terminal DS is required in effective breeding for drought tolerance. This requires knowledge on mechanism such as deep root system, increased partitioning coefficient and conservative water use without reducing the shoot biomass production. Several National and International Consultative Groups on International Agricultural Research (CGIARs) such as International Crops Research Institute for Semi-Arid Tropics (ICRISAT) and International Centre for Tropical Agriculture (CIAT) have breeding programs that have deployed several high throughput phenotyping platforms and strategies to enhance drought tolerance through morpho-physiological and biochemical traits such as root biomass, better water use, canopy temperature depression (CTD), lower leaf development. These have been reported to be associated with drought tolerance in chickpea (Vadez et al., 2012; Nayak, 2010; Kashiwagi et al., 2006).

The impact of various root traits on drought tolerance was found to be high under terminal DS environment, especially in environment where plants solely depend on the stored soil moisture (Ludlow and Muchow, 1990; Kashiwagi et al., 2006; Kashiwagi et al., 2005; Turner et al., 2001; Passioura, 2006; Wasson et al., 2012). Several studies showed that root traits such as deep rooting are related to drought tolerance in chickpea and best genotypes respond by increasing roots deeper in the soil profile (Silim and Saxena, 1993; Benjamin and Nielsen, 2006), common beans (Sponchiando et al., 1989), and soybeans (Kaspar et al., 1978) have enhanced productivity despite low precipitation. In chickpea, Kashiwagi et al. (2006) reported that root development contributes to seed yield under terminal drought conditions as it is noted that root density per se would help in the greater extraction of available soil water. Similar study by Zaman-Allah et al. (2011) showed that in chickpea, there was limited correlation between root length density and yield. In related studies, Kirkegaard et al. (2007) demonstrated through field-based direct root and soil water measurements, that a 30 cm rooting depth increase in root system can capture an extra 10 mm of deep soil water at the grain development stage and result in an extra yield of 500 Kg per hectare. In addition, large root system with greater root prolificacy and rooting depth was shown to influence not only transpiration through soil moisture utilization but also shoot biomass production, harvest index (HI) under terminal DS (Kashiwagi et al., 2006,2013; Zaman-Allah et al., 2011; Purushothaman et al., 2017). But on the contrary, a deeper and more profuse roots alone had been considered not that important for higher grain yields (Vadez et al., 2008) or as a needless biomass partitioning (Passioura, 1983) or as an unnecessary energy loss due to its vigorous respiration compared to the shoot system (Krauss and Deacon, 1994). In cowpeas, more profuse (higher root length density, RLD) and deeper root systems are often viewed as desirable traits for drought adaptation, using a root box method and best cultivars were shown to have a

higher root dry matter per unit of leaf area and a downward movement of roots indicating that they would invest more in deeper rooting for water capture (Matsui and Singh, 2003). In chickpea, greater root density deep into the soil profile and the larger proportion of fine roots compared with field pea and soybean resulted in better exploitation of water stored at lower soil depths (Kashiwagi et al., 2006). In related studies, Saxena (2003) has been using ICC 4958 as a check for root studies due to its greater degree of drought tolerance from its large root traits. Several findings have noted that high root mass has been of concern because the more the roots, the more their efficiency in absorption of water. This gives the plant more advantage in times when less moisture is available in the soil. Krishnamurthy et al. (2003) reported that large root biomass in a mini-core collection of ICRISAT chickpea germplasm had high correlation with drought tolerance and could be used as selection criteria in early generation during breeding.

Improving the resistance of seedlings to water-deficit stress has a two-fold benefit. The first and direct benefit is that it enables crop establishment through withstanding early season drought (Blum 1996; Passioura, 2012) that happens shortly after successful germination. Similarly, Shaxson and Barber (2003) noted that water from precipitation or irrigation can be lost in the form of crop respiration, soil evaporation and percolation into deeper soil layers. The second advantage is that water stress resistance at early stage can also be indicative of resistance at later growth stages (Comas et al., 2013), which makes root evaluation easier. Also plants can re-access the water that has gone into deep percolation only if they have long and vigorous root growth at early stage. However, many researchers warned the need to be cautious in extrapolating early-stage results for later stage resistance unless it is tested and proved in the field (Passioura, 2012; Wasson et al., 2012; Comas et al., 2013). Munns (2011) noted that root system vigour describes the variation in the rate of root growth that results in the capture of greater volumes of soil water and nutrients. Furthermore, a recent study in wheat re-analyzed the implication of root system size and water capture and concluded that, because of the close link between shoot growth and root growth, the development of a large root system might be better suited to environments where the crop depends on in-season rainfall like the Mediterranean environment, whereas under terminal stress conditions in semi-arid tropics of Asia and Africa, a vigorous root system that is linked to a vigorous shoot, would run the risk of a rapid water depletion of the soil profile and eventually a severe stress during reproduction and grain filling (Watt et al., 2005; Liao et al., 2006; Palta et al., 2011). Hence, two recent modelling studies illustrate this idea and a recent review argues that roots need to be looked at with a view to the whole plant and with a view to resource availability in time and space (Lynch, 2007; Sinclair et al., 2010; Vadez et al., 2012; Comas et al., 2013).

In response to this dilemma, many authors have reported that constitutive traits such as deep root system (Manschadi et al., 2006, Lilley and Kirkegaard, 2011), fine roots with small diameters, root length density (Blum, 2010; Comas et al. 2013), leaf rolling, leaf waxy layer and osmotic adjustment (Blum, 2010) are among the frequently studied traits that confer dehydration avoidance mechanism to plants. Blum (2010) furthermore reported that deeper roots allows the crop to access more water, maintain high stomata conductance and hence photosynthesis, and are indicated by cooler canopies. In this study, both root screening under rainout shelter and field screening at arid and semi-arid lands (ASALs) of Baringo County were conducted to confirm and prove the value and contributions of root traits to improving water use and productivity. The objective of the study is to assess the root variation in selected parental chickpea and identify the key root traits that could contribute to enhancing drought tolerance under water stress conditions semi-arid areas of East Africa.

MATERIALS AND METHODS

Site description

Egerton University, Njoro (0° 22'S, 35º 56'E; altitude of 2,238 m above sea level) has a mean day temperatures of 21 °C, and a mean annual rainfall of 900 to 1,020 mm which falls in a bimodal pattern, with long and short rains (Ondieki et al., 2013; Jaetzold and Schimdt, 1983).

Plant material evaluated

Ten parental genotypes were evaluated for root traits which included four released varieties in Kenya: Chania Desi 1 (ICCV 97105), LTD 068 (ICCV 00108), Chania Desi 2 (ICCV 92944) and a local germplasm commonly referred to as Ngara local. Three advanced lines (ICCV 92318, ICCV 97306 and ICC 3325), two susceptible checks (ICC 283 and ICC 1882) with poor rooting characteristics and ICC 4958 was used as the tolerant check due to its prolific and large root properties (Saxena, 2003). Yield data from field evaluations earlier conducted was included in the study. Table 1 describes the status of the tested plant materials.

Experiment description

The experiment was conducted in Egerton University Field 7 Research Station under rain-out shelter May/September 2013/2014 seasons and a second experiment was conducted during November, 2013/January, 2014 season. The experiment was set in Polyvinyl chloride (PVC) cylinders measuring 120 cm long and 20 cm diameter under rain out shelter. The cylinders were placed in 1.2 m deep cement pits with a spacing of 0.05 m between cylinders, giving a planting density of 20 plants m^{-2} and they were arranged in Randomized Completely Block Design (RCBD) in three replicates. The cylinders were filled with an equal mixture (w/w) of mollic-andosols (forest soil) and sand. The sand was used to decrease the soil bulk density and facilitate root growth and subsequent root extraction. Two seeds of each genotype were sown in the cylinder

Table 1. The status of the tested plant materials.

S/N	Genotype	Type	Status
1	Egerton Chania Desi1 (ICCV 97105)	Desi	Commercial check
2	Leldet 068 (ICCV 00108)	Desi	Commercial check
3	Egerton Chania Desi 2 (ICCV 92944)	Desi	Commercial check
4	ICCV 92318	Kabuli	Advanced breeding lines
5	ICC 4958	Desi	Drought tolerant check (High root length)
6	ICCV 97306	Kabuli	Advanced breeding lines
7	ICC 3325	Desi	Breeding line
8	ICC 283	Desi	Susceptible Breeding line
9	ICC 1882	Kabuli	Susceptible line (Low root length)
10	Ngara local	Desi	Tolerant local accession
11	CAVIR	Kabuli	Spanish Tolerant variety

and irrigated with 2,000 ml water uniformly to achieve uniform emergence. At 14 days after sowing (DAS) water stress treatment was imposed and one seedling was thinned out. There were three water regimes which were imposed: high moisture (75% of near field capacity - FC), medium moisture (50% of near field capacity) and low moisture (25% of near field capacity). This was maintained till 40 DAS (end of vegetative growth). Every two alternate days, 1.5, 1.0, and 0.5 litres of water were used to replenish the high, medium and low moisture levels respectively. Initial calibration of the soil water to be used was done before planting to determine the water holding capacity which ranged between 0.28 to 0.48 cm^3 cm^{-3} lower limit-upper limit respectively for the 0 - 120 cm PVC pipe soil layer and the volume of the water added each time (Ooro et al., 2003; Kimurto et al., 2005). Weeding was done by physically uprooting weedy species once they had emerged.

Measurements on root and shoot traits

Roots were extracted from the PVC pipes by gently washing out the soil particles and other debris at the age of 35 days after sowing (DAS) from the lower end of pipe. When approximately three quarters of the soil–sand mixture was washed away, the cylinders were erected gently on a 2 mm sieve so that the entire root system could be removed. The extracted root system was mostly in one piece with very few small segments of detached roots trapped by the 5 mm sieve. The roots were thoroughly cleaned, separated from the organic debris and straightened by repeated dipping and rising in buckets of clear water, then floating the sample material on water in trays. The entire process was repeated for all the tubes and the roots were separated from the above ground biomass by cutting at the cotyledonary point and put in paper bags for oven for drying to a constant weight as earlier described (Purushothaman et al., 2017). Recovered roots were suspended in a transparent tray with 2 - 3 mm film of water for easy dispersion of roots before scanning. The root system was divided into segments of 15 cm which were placed in the scanning trays. Each root sample was measured using the image analysis system (Win-Rhizo, Regent Instruments INC., Quebec, Canada) following the methodology previously described by Serraj et al. (2004). The roots were kept for oven drying at 70°C for 72 h (to constant weight). The following traits were measured: 1) Shoot dry weight (SDW) (g) – Shoots separated from roots were oven dried at 800°C for 72 h and their weights recorded. The SDW was used as an indicator of plant growth vigour; 2) Root dry weight (RDW) (g) – Scanned roots were oven dried at 80 ºC for 72 h and their weights recorded. The RDW was used as an indicator for drought tolerance; 3) Root: shoot ratio (R:S) was calculated using root and shoot dry weights which was calculated as the ratio of roots dry weight to shoot dry weight; 4) Total dry weight (TDW) (g). This was calculated by combining the SDW and RDW; 5) Total rooting length (TRL) (cm) was measured using an image analysis system (WinRhizo, Regent Instruments Inc., Canada); 6) Specific root length (SRL) was determined by diving root length over root dry mass (RDM) in Mg^{-1} dry (RL/RDM), and 7) Root length density (RLD) was calculated as earlier described by Zaman-Allah et al. (2011) as RLD ($cmcm^{-3}$) = Length of roots (cm)/volume of soil core (cm3). The soil volume was calculated using the following mathematical expression:

Soil volume= π.r2xh,

Where; π = 3.14; r = Soil core inner radius (20 cm PVC pipe); h = Sub-core height (120 cm).

Data analysis

Data analysis was performed by GenStat (14th edition) statistical software. The means were separated by least significant difference at P < 0.05. The following statistical model was used:

$$Y_{ijk} = \mu + G_i + S_l + R_j + B_{(kj)} + GS_{il} + \varepsilon_{ijk}$$

Where: Y_{ijk}= observations; μ= mean of the experiment; G_i= effect of the i^{th} genotype; S_l= effect of l^{th} season; R_j= effect of the j^{th}replicate; $B_{(jk)}$= effect of the k^{th} in complete block within the j^{th}replicate; GS_{il}= effect of i^{th} genotype in l^{th} season and ε_{ijk}= experimental error. The least significant difference was determined at P < 0.05.

RESULTS

Effects of water treatments on root traits for test genotypes under rain shelter

The results for combined analysis of measured root traits showed that there were significant differences (P < 0.05) in the genotype and water treatments (25% low, 50%

Table 2. Mean squares for crop morpho-physiological traits linked to drought tolerance traits under various watering regimes at Egerton research station for season I and II 2014 season.

Source of variation		SDW		RDW		TDW	
	d.f.	SI	SII	SI	SII	SI	SII
G	9	1.90**	2.06***	0.22***	0.08	2.22**	2.69***
WT	2	3.1	0.97	0.28**	0.03	4.95**	0.96
G×WT	18	1.2	0.3	0.41***	0.02	2.22***	0.41
Season							
Error	58	0.59	0.37	0.05	0.03	0.73	0.48
Total	87						
CV%		13.7	9.3	2.4	7.2	10.4	8
l.s.d.0.05 G		0.72	0.58	0.2	0.16	0.8062	0.6562
l.s.d.0.05 WT		0.4	0.32	0.11	0.09	0.4416	0.3594
l.s.d.0.05 G×WT		1.25	1.01	0.35	0.28	1.3964	1.1366

Source of variation		R:S		TRL		RLD	
	d.f.	SI	SII	SI	SII	SI	SII
G	9	22.69*	15.72*	239898*	608663**	0.02*	0.07*
WT	2	9.69*	5.48*	233838*	62334*	0.02*	0.01*
G.WT	18	42.07	7.9	540100	173196	0.05	0.02
Season							
Error	58	12.7	14.86	324615	319953	0.03	0.033
Total	87						
CV%		20	17.4	11.2	12.8	11.2	12.9
l.s.d.0.05 G		7.472	4.705	537.6	533.8	0.02	0.02
l.s.d.0.05WT		4.092	2.577	294.5	292.3	0.01	0.01
l.s.d.0.05 G×WT		12.941	8.149	931.2	924.5	0.03	0.03

Level of significance: ***- 0.001, **- 0.05 and *-0.01, d.f.- degrees of freedom, SI- the first season, SII- the second season, SDW- Shoot dry weight, RDW- Root dry weight, TDW-total dry weight, R:S- Root: to shoot ratio, TRL- Total root length, RLD- Root length density, WT- water treatment, G- Genotype.

medium, and 75% high) (Table 2). Genotype and the interactions between genotype and water treatments and genotype and season affected all the root traits of tested chickpea germplasm. Most of these traits varied significantly amongst test genotypes. The significance of the main effects of genotype (G), water treatment (WT), and genotype × water treatment interaction (GWT) were measured at $P < 0.05$. The presence of G×S and G×WT for the traits indicated that the output of the traits varied across the seasons and moisture treatment (Table 2).

Effects of water regimes on shoot dry weight (SDW) among test chickpea genotypes

The overall means for each moisture treatment (low to high) across seasons (I & II) showed that drought stress (DS) reduced the shoot dry weight (Table 3). The interaction between water regimes and chickpea genotypes was significant ($P < 0.05$) on the effect of shoot biomass accumulation over growing period (Table 3). Overall, moisture stress reduced SDW by 66% under low moisture as compared to high moisture treatment in season I (2013) and by 71% in season II (2013/14) due to the early stage rainfall (long rainfall season) (data not provided) that could have raised RH and delayed stress built up in the rain-out shelter. Overall genotypes varied significantly in SDW both in 2013 and 2013/2014 (Table 3). The overall mean SDW for both seasons combined varied from 0.86 - 0.87 g (ICC 1882 and ICC 283, respectively) to 1.84 - 2.24 g (ICC 4958, ICCV 97306, and ICCV 92318, respectively). In season I it ranged from 0.90 g (ICC 283) to 2.24 g (ICCV 92318) as compared to 0.84 to 2.18 g in season II. There was variation from 0.34 g per plant (ICCV 92318) under low water regime to 2.88 g per plant (ICCV 92318) under high watering regime. Overall the mean SDW in the second season was 1.49 g which was lower than 1.57 g recorded in the season I.

On average, genotype ICCV 92318 attained the highest SDW in season I and season II (2.29 and 2.18 g,

Table 3. Combined means of shoot dry weight (g) under varying watering regimes for season I and II (2013/2014).

Genotype	Season I				Season II				Overall mean
	Low moist	Medium moist	High moist	Mean	Low moist	Medium moist	High moist	Mean	
ICCV 92944	1.52	1.97	2.01	1.83	1.24	1.61	2.06	1.64	1.74
ICCV 00108	1.51	1.61	1.85	1.66	1.48	1.54	1.71	1.58	1.62
ICCV 97105	1.28	1.53	2.05	1.62	1.28	1.45	1.97	1.57	1.59
ICC 4958	1.41	1.97	1.95	1.78	1.66	1.89	2.18	1.91	1.84
ICCV 97306	1.43	2.09	2.82	2.11	1.36	1.79	2.74	1.96	2.04
ICCV 92318	1.04	2.73	3.09	2.29	1.14	2.53	2.88	2.18	2.24
Ngara Local	0.47	1.17	1.64	1.09	0.34	1.19	1.4	0.98	1.04
ICC 1882	0.84	0.91	1.04	0.93	0.53	0.98	1.08	0.86	0.90
ICC 283	0.77	0.85	1.09	0.90	0.77	0.85	0.91	0.84	0.87
ICC 3325	1.28	1.41	1.74	1.48	0.98	1.24	1.56	1.40	1.44
Mean	1.16	1.62	1.93	1.57	1.08	1.51	1.85	1.49	1.53
CV%				10.2				9.3	
l.s.d.0.05 G	*	*	**		**	**	**		
l.s.d.0.05 WT	*	*	*		*	**	*		
l.s.d.0.05 G×WT	*	*	*		*	*	*		

Level of significance ***- 0.001, **- 0.05 and *-0.01, SI- the first season, SII- the second season, G- Genotype, WT- water treatment, G×WT- Genotype x water regime interaction; Moist- Moisture level.

respectively). Genotype ICC 283 and ICC 1882 attained the lowest shoot biomass in both seasons (0.87 - 0.98 g). Under medium and high moisture regimes, SDW was greater by 39.6 and 18.4% (season I) and 40.0 and 71% (season II), respectively, than low moisture regime. On average in both seasons combined, drought tolerant check (ICC 4958) had 101.71 and 111.30% more SDW than susceptible genotype checks ICC 1882 and ICC 283, respectively.

Regardless of moisture, on average, genotypes ICCV 92318, ICC 97306 and ICCV 92944 had 28.6, 2.7 and 2.6% higher SDW than drought tolerant check (ICC 4958) in season I. In season II, ICCV 92318 and ICCV 97306 respectively recorded 21.7 and 10.8% higher SDW than drought tolerant check (ICC 4958) (Figure 1).

On average, in both seasons SDW increased with the increase of the water moisture from 25 to 75% FC (low to high moisture regime). For example, genotype ICCV 92318 had increasing SDW with the increase of moisture. For season I the genotype recorded shoot biomass of 1.04, 2.73 and 3.09 g in the low, medium and high moisture regimes, respectively, in season I as compared to 1.14g, 2.53 g and 2.88 g in the second season respectively. This indicates that ICCV 92318 had better response (176%) to high moisture level. This is in contrast to ICCV 92944 which had lower significance change in SDW with the increase of moisture. For season I the genotype recorded 1.53, 1.97 and 2.01 g in the low, medium and high moisture levels as compared to 1.24, 1.61 and 1.64 g in the low, medium and high moisture levels, thus indicating that ICCV 92944 had low response (32%); increasing water supply thus can be adopted in regions with a low moisture level.

Effects of varying water regimes on total root biomass (RDW) among test chickpea genotypes

There was significantly large range of variations ($P < 0.05$) among the tested genotypes for average total root dry weight (RDW) measured during seedling stage in varied water treatments and seasons (Table 4). The interaction between water regimes and chickpea genotypes affected total root dry weight accumulation over growing period. Average RDW varied from 0.27 - 1.63 g in season I to 0.18 - 1.13 g in season II (Table 4). The overall mean RDW was 15% higher in season I (0.55 g) than season II (0.48 g) (Table 4). Moisture stress reduced RDW by 114% under low moisture as compared to high moisture treatment in season I (2013) and by 70% in season II (2013/14) as compared to 54 and 32% under low moisture as compared to medium moisture treatment in season I (2013) and season II (2013/14), respectively.

Under high moisture regimes, RDW was greater by 38.8% (season I) and 32.5% (season II) than medium moisture respectively. In season I, the drought tolerant check (ICC 4958) had 222 and 163% higher RDW than susceptible genotype checks ICC 1882 and Ngara local respectively. In season II, ICC 4958 had 126, 188 and 73% greater RDW than genotypes ICC 1882, ICC 3325 and Ngara local, respectively. Similar trends were observed under low and high moisture regions. Parental

Figure 1. Root traits of test genotypes showing differences in morphology before root scanning under low moisture regimes (25% FC).

Table 4. Mean of root dry weight (RDW) (biomass) (g) for the test genotype under varying watering regimes for season I and II (2013/2014).

Genotype	Season I				Season II				
	Low moist	Medium moist	High moist	Mean	Low moist	Medium moist	High moist	Mean	Overall mean
ICCV 92944	0.31	0.41	0.57	0.43	0.3	0.35	0.44	0.36	0.4
ICCV 00108	0.35	0.41	0.45	0.4	0.27	0.33	0.39	0.33	0.37
ICCV 97105	0.21	0.39	0.46	0.35	0.23	0.3	0.44	0.32	0.34
ICC 4958	0.41	0.54	1.66	0.87	0.45	0.54	0.57	0.52	0.7
ICCV 97306	1.21	1.74	1.93	1.63	0.91	0.94	1.55	1.13	1.38
ICCV 92318	0.26	0.57	0.72	0.52	0.27	0.55	0.67	0.5	0.51
Ngara Local	0.14	0.37	0.48	0.33	0.23	0.27	0.41	0.3	0.32
ICC 1882	0.21	0.28	0.31	0.27	0.16	0.25	0.27	0.23	0.25
ICC 283	0.16	0.32	0.38	0.35	0.14	0.31	0.36	0.27	0.31
ICC 3325	0.29	0.34	0.49	0.37	0.14	0.18	0.21	0.18	0.28
Mean	**0.35**	**0.54**	**0.75**	**0.55**	**0.31**	**0.4**	**0.53**	**0.41**	**0.48**
CV%				5.4				7.2	
l.s.d.0.05 G	*	**	**		**	**	**		
l.s.d.0.05 WT	*	**	*		**	*	*		
l.s.d.0.05 G×WT	*	*	*		*	*	**		

Key: Level of significance ***- 0.001, **- 0.05 and *-0.01, SI- the first season, SII- the second season, G- Genotype, WT- water treatment, G x WT- Genotype x water regime interaction; Moist-Moisture level.

test genotypes varied significantly in RDW both in 2013 and 2013/2014 (Table 4). The overall mean RDW for both seasons combined varied from the 0.25 - 0.28 g (ICC 1882 and ICC 3325 respectively) to 0.70 - 1.38 g (ICC 4958, ICCV 97306, respectively). In season I, RDW ranged from 0.27 g (ICC 1882) to 1.63 g (ICCV 97306) as compared to 0.18 g to 1.13 g in season II (Table 4).

The variation under low and medium water regime was 0.14-0.16 g per plant (Ngara local and ICC 283, respectively) to 1.66-1.93 g per plant (ICC 4958 and

ICCV 97306, respectively) under high watering regime (Table 4). In the second season lower values were recorded: ranging from the 0.41g (ICC ICC 3325) to 0.91 g (ICCV 97306) under lowest moisture regime as compared to 0.21 g per plant (ICC 3325) under low moisture to 1.55 g (ICCV 97306) under high moisture.

On average, genotypes ICCV 97306, ICC 4958, and ICCV 92318 had the highest root biomass (mean 0.86 g) in decreasing order in both seasons (1.38, 0.70 and 0.51 g, respectively), while ICC 1882, ICC 3325, ICC 283, and Ngara local had the lowest root biomass (mean 0.29 g per plant). Commercial varieties ICCV 92944, ICCV 00108, and ICCV 97105 had medium to high root biomass (0.39 g per plant) which was 121% lower than the best performing genotypes and 34% better than worst performing genotypes (Table 4).

Genotype ICCCV 97306 had the highest root dry biomass (mean 1.38 g per plant). This was higher than the drought tolerant check (ICC 4958) by 97 % in both seasons combined and by 87% (season I) and by 117% (season II). Across them moisture treatments the RDW of most test genotypes was increasing with the increase of water level, but was highest for ICC 283, Ngara local and ICCV 92318 which ranged from 97 - 155% RDW increase with increasing moisture from low to highest moisture in both season combined.

Effects of varying water regimes on total dry weight (TDW) (root and shoot biomass) among test chickpea genotypes

The interaction between water regimes and chickpea genotypes affected total dry weight (TDW) at root harvest measured at seedling stage (35DAE) ($P<0.01$), with significant range of variations among the tested genotypes in varied water treatments and seasons. The overall TDW increased with increasing moisture (25% FC) to 75% FC) with mean root and shoot biomass being 5.5% higher in season I (2.12 g) than season II (2.01 g). Overall moisture stress reduced TDW by 76% under low moisture as compared to high moisture treatment in season I (2013) and by 24% in season II (2013/2014) as compared to 43 and 21% under low moisture as compared to medium moisture treatment in season I (2013) and season II (2013/2014), respectively. Overall, the total shoot and root biomass (TDW) varied from the 1.41 g (ICC 1882) to 3.42 g (ICCV 97306). The mean TDW in the season I was 5.1% higher (2.12 g) than that recorded in the season II (2.01 g).

Test genotypes varied significantly in total shoot and root biomass (TDW) in both seasons (2013/2014). The overall mean TDW for both seasons combined varied from 1.20 - 1.25 g (ICC 1882 and ICC 283, respectively) to 2.65 - 3.74 g per plant (in season I) to 1.14-1.18 g for same genotypes to 2.54, 2.74 and 3.42 g for genotypes ICC 4958, ICCV 92318 and ICCV 97306 respectively in season II (Table 4). In season I, TDW ranged from 0.91 g (ICC 283) to 3.74 g (ICCV 97306) as compared to 0.69 g to 3.36 g in season II.

There was great variation between moisture regimes (25% FC - 75% FC). In first season, under low water regime TDW was lowest ranging from 0.61-0.91 g per plant (Ngara local and ICC 283, respectively) to 3.61, 3.81 and 4.75 g per plant (ICC 4958, ICCV 92318 and ICCV 97306, respectively) under high watering regime (Table 4). As compared to the second season lower values were recorded: ranging from the 0.69 g (ICC 1882) to 2.08, 2.79 and 2.73 g for genotypes ICC 4958, ICCV 92318 and ICCV 97306, respectively (Table 5). Overall the genotype ICCV 97306 had the highest TDW in both seasons combine (3.42 g) while genotype ICC 1882 had the lightest shoot and root weight (1.14 g) followed by ICC 283 (1.18 g) which was 200% and 189% higher, respectively (Table 4). Similar trends were recorded for season I and II

In both seasons, the drought tolerant genotype ICC 4958 had below the average mean (2.54 g) of the two best performing genotypes (ICCV 97306 and ICCV 92318) which had the greatest TDW (3.08 g), 21% lower (Table 4). Drought susceptible genotypes ICC 1882, ICC 3325 and ICC 283 consistently had low TDW under low moisture, medium, and high moisture, respectively. Drought tolerant check (ICC 4958) had 108% greater TDW (mean 2.54 g) than the mean of three susceptible genotype checks (mean 1.22 g) (ICC 1882, Ngara local, and ICC 283) as compared to 152% greater TDW for three best performing genotypes (ICCV 97306 and ICCV 92318) (mean 3.08 g). Regardless of the moisture level, these two genotypes recorded the highest mean TDW in both seasons (3.27 g and 3.08 g respectively). This was higher than the drought tolerant check (ICC 4958) by 21.2% (Table 4). Overall, TDW of most test genotypes was increasing with the increase of moisture applied from 25 - 75% FC, but response varied with highest recorded for genotype ICC 92318 in both seasons combined (Table 4).

Effects of varying water regimes on total root length (TRL) among test chickpea genotypes

The interaction between water regimes and chickpea genotypes significantly ($P < 0.05$) affected total root growth over the seedling stage growing period (Table 6). The overall means for each moisture treatment (low to high: 25 - 75% FC) across seasons I and II showed that moisture stress reduced the total root length by 61.4% from 1.64 to 1.02 m from high to low moisture in both seasons combined (Table 6). Similarly, moisture stress reduced TRL by 28.8% (1.31 m) from medium (50% FC) to low moisture (25% FC) (Table 6). This varied with seasons: under low moisture TRL decreased by 65.7% as compared to high moisture treatment in season I (2013)

Table 5. Mean of on Total dry weight (TDW) (root and shoot biomass) (g) for the test genotype under varying watering regimes for season I and II (2013/2014).

Genotype	Season I				Season II				
	Low moist	Medium moist	High moist	Mean	Low moist	Medium moist	High moist	Mean	Overall mean
ICCV 92944	1.83	2.38	2.58	2.26	1.54	1.96	2.5	2	2.13
ICCV 00108	1.86	2.02	2.3	2.06	1.81	1.87	1.89	1.91	1.98
ICCV 97105	1.49	1.92	2.51	1.97	1.51	1.75	1.84	1.89	1.93
ICC 4958	1.82	2.51	3.61	2.65	2.4	2.43	2.08	2.43	2.54
ICCV 97306	2.64	3.83	4.75	3.74	1.76	2.73	2.79	3.1	3.42
ICCV 92318	1.3	3.3	3.81	2.8	1.7	3.08	3.36	2.68	2.74
Ngara Local	0.61	1.54	2.12	1.42	1.63	1.46	1.34	1.28	1.35
ICC 1882	1.05	1.19	1.35	1.2	0.69	1.23	1.35	1.09	1.14
ICC 283	0.91	1.17	1.47	1.25	1.48	1.16	1.21	1.11	1.18
ICC 3325	1.57	1.75	2.23	1.85	1.2	1.42	1.15	1.58	1.71
Mean	1.51	2.16	2.67	2.12	1.57	1.91	1.95	1.91	2.01
CV%					11.4				9.62
l.s.d.0.05 G	*	*	*		**	*	*		
l.s.d.0.05 WT	*	*	*		*	*	*		
l.s.d.0.05 G×WT	ns	*	*		ns	*	*		

Key: Level of significance ***- 0.001, **- 0.05 and *-0.01, SI- the first season, SII- the second season, G- Genotype, WT- water treatment, G×WT- Genotype x water regime interaction; Moist- Moisture level.

Table 6. Mean of Total root length (TRL) (cm) for the test genotype under varying watering regimes for season I and II (2013/2014).

Genotype	Season I				Season II				
	Low moist	Medium moist	High moist	Mean	Low moist	Medium moist	High moist	Mean	Overall mean
ICCV 92944	953	1475	1705	1377.7	921	1324	1694	1313	1345.3
ICCV 00108	1162	1380	1778	1440	916	1313	1445	1224.7	1332.3
ICCV 97105	879	1201	1498	1192.7	840	1025	1562	1142.3	1167.5
ICC 4958	1426	1680	1973	1693	1396	1592	1639	1542.3	1617.7
ICCV 97306	1029	1498	2282	1603	1032	1348	2009	1463	1533
ICCV 92318	1020	1329	1856	1401.7	1022	1502	1836	1453.3	1427.5
Ngara Local	1055	1247	1591	1297.7	895	945	1132	990.7	1144.2
ICC 1882	1071	1205	1521	1265.7	950	1029	1090	1023	1144.3
ICC 283	870	1129	1570	1189.7	869	1145	1549	1187.7	1188.7
ICC 3325	1125	1517	1775	1472.3	958	1390	1418	1255.3	1363.8
Mean	1059	1366.1	1754.9	1393.33	979.9	1261.3	1537.4	1259.5	1326.4
CV%				18.4				19.62	
l.s.d.0.05 G	*	**	**		**	*		**	
l.s.d.0.05 WT	*	**	*		*	*		**	
l.s.d.0.05 G×WT	*	*	*		**	*		*	

Key: Level of significance ***- 0.001, **- 0.05 and *-0.01, SI- the first season, SII- the second season, G- Genotype, WT- water treatment, G×WT- Genotype x water regime interaction; Moist- Moisture level.

and by 28.7% in season II (2013/14). This could be due to the early rainfall during the long season rainfall (March-May) (data not provided) that could have raised RH and delayed stress built up in the rain-out shelter as compared to delayed and shorter season rainfall at Egerton during second season (Oct-Feb).

There was significant variation ($P < 0.05$) in TRL among test genotypes both in 2013 and 2013/2014 (Table 6). The

overall mean TRL for both seasons combined varied from 1144.2-1167.7 cm (Ngara local and ICCV 97105, respectively) to 1427.5 cm (ICCV 92318, ICCV 97306, and ICC 4958, respectively). In season I TRL ranged from 1189.7cm (ICC 283) to 1693.0 cm (ICC 4958) as compared to 990.7 cm to 1617.7 cm in season II (Table 6). In season I, TRL varied from 870.0 cm (ICC 283) under low water regime to 2282.0 cm (ICCV 97306) under high watering regime (Table 6) as compared to 869.0 cm (ICC 283) under low moisture to 2009 cm (ICCV 97306 under high moisture in second season. Overall the mean TRL in the second season was 1295.5 cm which was 11% lower than recorded in the season I (1393.3 cm).

On average, TRL increased with the increase in soil moisture from low to high moisture regime. For example, in season I, the mean TRL recorded was 1059, 1366.1 and 1754.9 cm in the low, medium and high moisture regimes, respectively, as compared to 979.9, 1261.3 , and 1537.4 cm in the low, medium and high moisture levels, respectively, in second season. In both seasons, the drought tolerant genotype, ICC 4958 had above average mean (1617.7 cm) of the two best performing genotypes (ICCV 97306 and ICCV 92318) which had the longest TRL (1533 cm and 1427.5 cm), which was respectively 6 and 13% higher (Table 6). Drought susceptible genotypes ICC 1882, ICC 3325, ICC 283, and Ngara local consistently had low TRL under low moisture, medium and high moisture, respectively. Drought tolerant check (ICC 4958) had 35% greater TRL (mean 1617.7 cm) than the mean of four worst performing susceptible genotype checks (mean 1200.9 cm) (ICC 1882, Ngara local, ICC 3325, and ICC 283). In contrast ICC 4958 had 12% higher TRL than the best performing tolerant genotypes (ICCV 97306, ICCV 92318, and ICCV 92944) with mean of 1435.1 cm.

Regardless of the moisture level, these three genotypes recorded the highest mean TRL in both season combined (1533 cm and 1427.5 and 1345.3 cm, respectively. Consistently, genotypes ICCV 97105 and ICCV 00108 had unexpectedly shorter roots (mean 1249.9 cm) than the drought tolerant check (ICC 4958) and best performing commercial checks by 29.4 and 14.8%, respectively. Overall, TRL of most test genotypes was increasing with the increase of moisture applied from 25 - 75% FC, but response varied with highest (121%) recorded for genotype ICC 97306 in both seasons combined. This shows that this genotype had highest response to increasing water supply.

Effects of varying water regimes and chickpea genotypes on root:shoot (R:S)

The interaction between water regimes and chickpea genotypes significantly ($P < 0.05$) affected root: shoot root at harvest (Table 7). Overall, water regimes from low to high (25 - 75% FC) had non-significant increase in R:S ratio under low moisture, but increased R:S ratio by 26% from 0.306 to 0.386 from low to high moisture in season I as compared to 2.5% from 0.286 to 0.287 from low to high moisture in season II (Table 7). The mean R:S ratio in the season I was 0.352 which was higher than 0.278 recorded in the season II (Table 7).

In both seasons (2013 and 2013/2014), there was significant variation ($P < 0.05$) in R:S ratio among test genotypes (Table 7). The overall mean R:S ratio for both seasons combined varied from the 0.190 to 0.212 (mean 0.201) (ICC 3325 and ICCV 97105, respectively) to 0.673 (ICCV 97306 and ICC 4958, respectively). Similarly, in season I, R:S ratio ranged from 0.218 (ICCV 97105) to 0.770 (ICC 4958) as compared to 0.126 to 0.577 (ICC 3325 and ICCV 97306, respectively) in season II (Table 7).

Under low water regime genotypes ICC 3325, ICCV 97105, and ICCV 92944 had lowest R:S ratio while genotypes ICCV 97306, ICC 4858, and Ngara local had highest R:S ratio. Similar trends were observed under medium and high watering regime (Table 6). Overall the mean R:S ratio in the second season was 0.278 which was 35% lower than that recorded in the season I (0.386). On average, genotype ICCV 97105 had the lowest average R:S ratio amongst commercial genotypes in both season (0.212) while ICC 3325 had the lowest average R:S in both seasons (0.180).

Drought tolerant check (ICC 4958) and best performing genotype (ICCV 97306) had 146% R:S ratio (mean 0.63) than worst performing susceptible genotypes (mean 0.256) (ICC 1882, ICC 3325, and ICCV 92318). The increase in R:S ratio of most test genotypes was not consistent with increase in moisture applied from 25 - 75% FC as most traits measured. The highest response was recorded for genotype ICC 97306 in both seasons combined, showing that this genotype had highest response to increasing water supply.

Effects of varying water regimes and chickpea genotypes on root length density (RLD)

The interaction between water regimes and chickpea genotypes did not affect root length density, but there were significant differences between test genotypes across water regimes (Table 8). The overall means for each moisture treatment (low to high: 25 - 75% FC) across seasons I and II showed that RLD reduced with increasing moisture stress. In both seasons combined, RLD was reduced by 34.5% when moisture was decreased from high to low moisture regime. Similarly in season I, RLD was reduced by 35.4% (from 0.218 to 0.161 cm cm^{-3}) under medium to low moisture regime as compared to 33% (0.290 cm cm^{-3} to 0.218 cm cm^{-3}) under high to medium moisture. In the same way, in season II, moisture stress reduced RLD by 27.5 and 30%

Table 7. Mean of root:shoot (R:S) ratio under varying watering regimes for season I and II (2013/2014).

Genotype	Season I				Season II				
	Low moist	Medium moist	High moist	Mean	Low moist	Medium moist	High moist	Mean	Overall mean
ICCV 92944	0.204	0.208	0.284	0.235	0.242	0.217	0.214	0.222	0.228
ICCV 00108	0.232	0.255	0.243	0.243	0.182	0.214	0.228	0.209	0.226
ICCV 97105	0.164	0.255	0.224	0.218	0.18	0.207	0.223	0.206	0.212
ICC 4958	0.291	0.274	0.851	0.49	0.271	0.286	0.261	0.272	0.381
ICCV 97306	0.846	0.833	0.684	0.77	0.669	0.525	0.566	0.577	0.673
ICCV 92318	0.25	0.209	0.233	0.226	0.237	0.217	0.233	0.227	0.227
Ngara Local	0.298	0.316	0.293	0.302	0.676	0.227	0.293	0.311	0.306
ICC 1882	0.25	0.308	0.298	0.287	0.302	0.255	0.25	0.263	0.275
ICC 283	0.182	0.376	0.349	0.387	0.182	0.365	0.396	0.32	0.354
ICC 3325	0.227	0.241	0.282	0.253	0.143	0.145	0.135	0.126	0.19
Mean	0.306	0.331	0.386	0.352	0.286	0.267	0.287	0.278	0.315
CV%			10.28					9.86	
l.s.d.0.05 G	*	**	**	**	**	*			
l.s.d.0.05 WT	**ns**	****	*****	**ns**	*****	*****			
l.s.d.0.05 G×WT	*	*	*	*	**	*			

Key: Level of significance ***- 0.001, **- 0.05 and *-0.01, ns-non-significant, SI- the first season, SII- the second season, G- Genotype, WT- water treatment, G×WT- Genotype ×water regime interaction; Moist- Moisture level.

Table 8. Mean of root length density (RLD) (cm cm^{-3}) under varying watering regimes for season I and II (2013/2014).

Genotype	Season I				Season II				
	Low moist	Medium moist	High moist	Mean	Low moist	Medium moist	High moist	Mean	Overall mean
ICCV 92944	0.16	0.215	0.345	0.24	0.17	0.215	0.255	0.213	0.227
ICCV 00108	0.15	0.25	0.3	0.233	0.2	0.2	0.25	0.217	0.225
ICCV 97105	0.1	0.2	0.25	0.183	0.15	0.15	0.25	0.183	0.183
ICC 4958	0.2	0.25	0.3	0.25	0.1	0.25	0.3	0.217	0.233
ICCV 97306	0.1	0.25	0.35	0.233	0.2	0.2	0.325	0.242	0.238
ICCV 92318	0.15	0.2	0.3	0.217	0.15	0.225	0.275	0.217	0.217
Ngara Local	0.1	0.15	0.2	0.15	0.105	0.17	0.195	0.157	0.153
ICC 1882	0.15	0.18	0.275	0.202	0.1	0.15	0.25	0.167	0.184
ICC 283	0.25	0.23	0.28	0.253	0.2	0.205	0.215	0.207	0.23
ICC 3325	0.2	0.22	0.27	0.236	0.18	0.226	0.25	0.229	0.228
Mean	0.161	0.218	0.29	0.223	0.156	0.199	0.259	0.204	0.214
CV%				13.28					
l.s.d.0.05 G	*	*	*		*	*		*	
l.s.d.0.05 WT	*	*	*		*	*		*	
l.s.d.0.05 G×WT	ns	*	ns		*	ns		*	

Key: Level of significance ***- 0.001, **- 0.05 and *-0.01, S I- the first season, S II-the second season, G- Genotype, WT- water treatment, G×WT- Genotype × water regime interaction; Moist- Moisture level.

under low to medium moisture and from high to medium moisture respectively, indicating that genotypes responded almost uniformly to decreasing moisture; however there was a higher decrease season I than season II (Table 7).

The average RLD of genotypes evaluated varied from 0.153 to 0.184 cm cm^{-3} (Ngara local, ICCV 97105, and ICC 1882) to highest RLD of 0.228 to 0.238 cm cm^{-3} (ICC 3325, ICC 4958 and ICCV 97306) in both seasons combined (Table 8). Except for genotype ICCV 97105,

other commercial checks (ICCV 00108 and ICCV 92944) had 3 and 5.3% lower RLD that drought tolerant check (ICC 4958) and best performing genotype (ICCV 97306), respectively (Table 8).

In both seasons, RLD increased with increase in water regime, but with significant differences between seasons and moisture regimes. On average, genotypes ICCV 97105, Ngara local, and ICC 1882 had the lowest average RLD (mean 0.173 cm cm^{-3}) in both seasons followed by ICCV 92944, ICCV 00108, ICCV 92318, ICC 283, and ICC 3325 (mean 0.225 cm^{-3}), while genotypes ICC 4958 and ICCV 97306 had highest RLD (mean 0.235 cm cm^{-3}). However, in both seasons, ICCV 97306 and ICC 4958 recorded the highest RLD (Table 8).

DISCUSSION

The findings of this study showed that root (and shoot) traits measured had good range of variation among the test chickpea genotypes under the three varied moisture regimes. This is to some extent in agreement to previous studies under both field and lysimetric conditions (Purushothaman et al., 2017; Serraj et al., 2004; Kashiwagi et al., 2005; Lalitha et al., 2015). Genotype and the interactions between genotype and moisture treatments affected most of the root traits (shoot dry weight, root dry weight, total dry weight, root:shoot ratio, total root length and root length density) of tested chickpea germplasm. As expected, increasing moisture stress through reducing moisture supply from high moisture to low moisture (75 - 25% field capacity) reduced most of the measured traits. For example SDW, RDW, TRL, and RLD was reduced by 68, 92, 61 and 34.4% under low moisture as compared to high moisture treatment in both seasons combined, with higher effect in season two than season one. This was probably because of early stage rainfall (long rainfall season) (data not provided) that could have raised RH and delayed stress built up in the rain-out shelter in season one. There was however non-significant effect on R:S ratio under varying moisture indicating that under the water stress levels of this study, the test genotypes could not show significant investments to roots than shoots. Generally, these root traits have clearly differentiated the drought tolerant genotypes from the sensitive ones, and explained why tolerant genotypes have better soil water acquisition under drought stress field conditions. This was earlier demonstrated by these genotypes (ICCV 97306, ICC 4958, and ICCV 92944) which produced higher yields in Chemeron and Marigat in Baringo (Muriuki et al., 2018). For example genotype ICCV 97306 outperformed the tolerant check (ICC 4958) in most root traits measured (RLD, TRL, RDW, and R:S ratio), while susceptible checks (ICC 1882 and ICC 3325) recorded lower root traits values across water regimes. These findings are in agreement with those earlier reported by Lynch (2007) and Purushothaman et al. (2017) who noted that root architecture is critically important for soil water acquisition and most of the tolerant chickpea genotypes had displayed root growth vigor and deeper soil root proliferation at early to mid-growth period for better adaptation to drought.

They also noted that architectural traits such as basal-root gravitropism (vertical root growth angle), adventitious-root formation (RLD) and lateral branching would offer the advantage in terms of the competition in photosynthate allocation between shoot and root growth and would lead to deep root systems (TRL) without overtly changing root biomass allocation. From this study drought genotypes ICC 4958 and ICCV 97306 had highest RLD (mean 0.235 cm^{-3}), above average mean TRL (1575 cm) and highest root dry mass (RDW) while drought susceptible genotypes (ICC 1882, ICC 3325 and ICC 283, and Ngara local) consistently recorded lower values. Hence one of the options to improve the root systems for drought avoidance is the enhancement of root growth vigour leading to deeper root penetration as shown by the two best performing genotypes. Rooting growth at different depth was however not measured in this study. This suggests that these morpho-physiological root traits (especially TRL) could be used as indirect selection criteria to augment yield-based selection procedures done under field condition. Similarly, Kashiwagi et al. (2005) and Gregory (1988) reported the existence of a large diversity in chickpea rooting depth which ranged from 88 to 126 cm at 35 DAS under long PVC cylinder culture conditions in ICRISAT and from 60 to 150 cm at crop maturity under field conditions respectively.

They observed that their studies also confirmed that previously known drought-tolerant chickpea genotypes such as ICC 4958 possess deep rooting ability. This is in agreement with the findings of this study where drought tolerant check (ICC 4958) had 35% greater TRL (mean 1617.7 cm) than the mean of four worst performing susceptible genotype checks (mean 1200.9 cm) (ICC 1882, Ngara local, and ICC 3325) at 35 DAS under long PVC cylinder culture conditions. These results show that precise targeting of root traits as indicators of yield would consequently lead to faster rates of yield improvement and broadening of genetic base under drought stress in ASALs. This is because, as compared to field evaluation done in several multi-locations, these traits are easier and faster to measure under rain out shelter than grain yield and they can also be observed at/or before flowering (seedling stage) and eliminate susceptible lines from crossing nursery and shorten the time to complete selection cycle. An estimate of yield potential under drought stress simulated conditions can therefore be determined more easily before final harvest.

This is in agreement with Lynch (2007) and Purushothaman et al. (2017) who proposed that breeding for the best combination of root traits mainly profuse RLD

at surface soil depths and RDW at deeper soil layers to be the best selection strategy for an efficient water use and an enhanced terminal drought tolerance in chickpea. In addition, Reynolds and Hunter (2001) noted that in wheat, a deliberate selection with a view to combining synergistic root traits like dry root weight, early seedling vigour, and RLD is likely to achieve results sooner than using grain yield performance alone.

Furthermore, Kashiwagi et al. (2006) suggested that rooting depth, root biomass, and root length density were identified as most promising traits in chickpea for terminal drought tolerance, as these help in greater extraction of soil moisture. More profuse (higher root length density, and RLD) and deeper root systems are often viewed as desirable traits for drought adaptation. In related legume cowpea crop, Matsui and Singh (2003), reported that tolerant genotypes had higher root dry matter per unit of leaf area and a downward movement of roots while using root pin box method, indicating that they would invest more in deeper rooting for water capture. As in this present study, the possible role of water extraction traits was demonstrated in that study by deeper rooting and higher root length density under decreasing drought stress from low to high moisture regime (25 to 75% field capacity). In related study, Kashiwagi et al. (2006) reported that chickpea genotypes reaching higher yield under terminal stress condition had higher RLD and genetic variability for root penetration rate of 2.5-3.6 cm day^{-1} and RLD of 0.19 -0.30 cm^{-3} among the chickpea mini-core germplasm collection (n = 211) at 35 DAS in similar tall cylinder culture systems (with 120 cm in height and 1.1 g cm^{-3} of bulk density) under rain-fed conditions. For sub-optimal complete extraction of soil moisture, RLD values of <0.5 cm cm^{-3} and <0.4 cm cm^{-3} particularly in Asia have been reported in lysimeters experimentation but even lower values have been reported which range from 0.150-0.252 cm cm^{-3} comparable to the findings of Kashiwagi et al. (2013).

In several related studies, Krishnamurthy et al. (1996), Kashiwagi et al. (2005) and Upadhyaya and Ortiz (2001) reported that genotypes ICC 4958 and ICC 8261 have been identified as the most prolific and deep-rooting chickpea accessions and they have been utilised as breeding materials to introgress these advantageous root traits into well-adapted regional chickpea cultivars for further improving grain yield under drought in semi-arid tropics (Kosgei, 2015; Varshney et al., 2014; Kimurto et al., 2017). In this present study, genotype ICCV 97306 has been identified as most prolific and deep rooting chickpea candidate similar to already identified ICC 4958 and could be used as donor for introgressing these water-uptake enhancing root traits into well-adapted chickpea cultivars for further improving grain yield under drought in semi-arid tropics.

These genotypes could be possessing larger xylems and phloem hence less capillary forces and low hydraulic resistance to water movement from soil to plant tissues through roots, thus increasing more soil water uptake and transport even under dry soils as compared to susceptible checks like ICC 3325 and ICC 1882. In related studies, Purushothaman et al. (2014) and Li et al. (2009) reported that chickpea has been shown to possess the largest number of xylem vessels among 6 major legume crops, hence a largest total xylem passage for water flow of 722 μm^2 in a single chickpea root as compared to 681 μm^2 in cowpea. They however noted that had the narrowest average diameter of 9.5 µm as compared to 14.0 µm in common bean. They noted that root systems with thin xylem vessels can be expected to have more capillary forces and less cavitation, and these are advantageous in terms of soil water uptake and transport even under dry soils. Similarly, Benjamin and Nielsen (2006) noted that as compared to other legumes like beans, chickpea also had relatively large xylem quantity and root biomass and the crop was expected to absorb more plant available soil water (PAW). These indicate that chickpea is more adapted to dense heavier soils in dry lands as compared to common beans.

In this study, it was also noted that most of the genotypes that possessed advantageous root traits and best performing (e.g. RLD of 0.228-0.238 $cmcm^{-3}$, TRL and RDW) were Desi genotypes (ICC 4958, ICCV 00108, ICCV 92944, and ICCV 92318) except for genotype (ICCV 97105-Desi) and (ICCV 97306-Kabuli) in both seasons combined. These indicate that the root anatomy could be varied among the two major chickpea types of the Desi (brown seed coat in smaller size) and the Kabuli (white seed coat in bolded larger seed size); the Desi could be having restrictive xylem and phloem vessels which could be conservative in water movement into and out of the tissues. This would lead to possible reduction in water loss due to transpiration and increase their performance in limited supply as shown under low and medium moisture regime (50 - 25% FC) compared to Kabulis which had better and higher response under high moisture regime in this study. In earlier studies, Desis had been reported to possess a moderate water uptake when compared to Kabulis, and considered conservative in their water requirement; they adapt well to the receding soil moisture environments than the Kabulis that had access to more water during the major part of their early growth (Berger et al., 2004).

Similarly, Purushothaman et al. (2014) noted that the xylem vessels in Desi were reported to be fewer in number and narrower in diameter compared to the Kabulis which he noted might explain why Desis had a moderate water uptake when compared to Kabulis; they were considered conservative in their water requirement adapting well to the receding soil moisture environments than the Kabulis. Several other studies also show an advantage of having superior root traits for yield under stress conditions (Silim and Saxena, 1993; Price et al., 2002b; Ober et al., 2005; Sarker et al., 2005; Tuberosa et al., 2002; Gowda et al., 2011). One of the important

mechanisms of drought avoidance is the ability of the plant to change its root distribution in the soil and this would vary by cultivar within a species (Benjamin and Nielsen, 2006). Genotype ICCV 92944 recorded best performance across seasons (stability) for this traits and hence indication of better adoption to drought condition.

Furthermore, Kashiwagi et al. (2005) observed cooler leaf canopy temperature estimated by infrared digital thermography at 70 DAS had a significant positive association with seed yield under terminal drought in field-grown chickpea at ICRISAT. This indicates that chickpea genotypes with greater transpiration at this stage would have greater reproductive growth leading to better seed yield under drought environments. They noted that although clear correlations were not consistently detected between leaf canopy temperature and root characteristics at 35 DAS, genotype ICC 4958 (drought tolerant check in this study) recorded a high prolific and deep root system and was one of the most highly transpiring leaf canopies among 16 diverse entries. In other studies, Vadez (2014) noted that in peanut (groundnuts) higher yields were obtained in where more profuse roots in the deeper soil layer were reportedly correlated to higher yield under water stress conditions, indicating that higher root length density (RLD) at depth was responsible for more water extraction. In contrast, drought stress strongly inhibited root growth of chickpea and that root growth ceased after the third week of stress (Tilahun and Sven, 2003). Previous work by Thomas (1995) reported that chickpea plants were found to have lower root length density than barley, but absorbed water more efficiently than barley plants. Amede and Schubert (2003) thus concluded that drought resistance of chickpea was due to the effect of osmotic adjustment, a function of root hydraulic conductivity, which is governed by the diameter and distribution of the meta-xylem vessels of the roots

Conclusion

The findings of this study showed that:

(i) Use of root traits to identify drought tolerance in chickpea during early growth stage significantly contribute to the seed yield in chickpea.
(ii) Increasing rooting depth, root biomass and RLD could increase the uptake of water and yield in chickpea which could be due to relatively large number of xylem vessels and root biomass which enhances better absorption of more plant available soil water (PAW) and superior adaptation to dense heavier soils in dry lands as compared to common beans. There was however variations in root anatomy between the two major chickpea types where majority of the best performing genotypes under low moisture regimes were of the Desi type (e.g. ICC 4958, ICCV 00108, ICCV 92944, and ICCV 92318) as compared to Kabulis which had better and higher response under high moisture regime in this study.
(iii) Root depth, root biomass and RLD can be used for indirect selection for drought tolerance especially in early stages of breeding for drought tolerance which would consequently reduce the cost of multi-location field evaluation in the breeding programs.
(iv) Genotype ICCV 97306 was identified as most prolific and deep rooting chickpea candidate similar to already identified tolerant check ICC 4958 and could be used as donor for introgressing these water-uptake enhancing root traits into well-adapted chickpea cultivars for further improving grain yield under drought in semi-arid tropics.
(v) Root and shoot growth is closely linked as shown by most genotypes and deeper rooting might lead to faster soil water depletion, which would be a problem for crops depending on stored soil moisture. Hence capturing deep layer water though metabolically expensive is a one-time benefit since any rainfall/irrigation event would wet the profile from the top in progressive drought stress conditions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Indoor characterization of three durum wheat genotypes exposed to drought and heat stress during early vegetative growth stages

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Selection of wheat varieties that have improved adaptation to abiotic stress is important for increasing and stabilizing yields under fluctuating environmental conditions, especially as global climate changes. A trial to estimate adaptation of wheat (*Triticum turgidum* subsp. *durum*) genotypes to abiotic stress has been performed, in a growth chamber. By counting the number of dead (yellow) plants, together with yellow and green leaves, and hence traits that easily can be also detected by automatized phenotyping platforms, were analyzed for the effects of optimal watering, progressive water deficit and different levels of heat stress. "Trinakria" variety and two Trinakria mutants ("Water-mutant" and "Hg-mutant") altered for water-related physiological traits were examined. The use of very genetically close genotypes had the aim to minimize differences in stress response due to asynchronous phenological development and to evaluate better the protocol usefulness to detect minimal phenotypic differences, such as those found between advanced breeding lines, at the final stages of a breeding program. Results showed that Trinakria had a significantly greater % of green leaves under drought stress and retained green leaf after heat stress ceased. In contrast, the two mutants had improved plant survival after moderate heat stress. In conclusion, an examination of leaf color changes under moderate water deficit and heat stress was sufficient in a differential comparison of genotypic performances.

Key words: Abiotic stress, leaf color, phenotyping, wheat.

INTRODUCTION

Despite world-wide efforts to select high yielding varieties, a decline in wheat production has been observed from the beginning of this millennium; mainly due to a lack of varieties that resist abiotic stress (Dalal et al., 2017). Changes in weather and climate, probably related to global warming, are shown by an increasing incidence of extreme weather phenomena, even during phenological phases in which the problem of dehydration stress was rare. At the vegetative phase, dehydration stress can modify the growth and development of plants

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(Wittmer et al., 1982; Figueroa-Bustos et al., 2019), so affecting up to 56% of the final yield (Gallagher et al., 1976). According to the concepts advanced by Negin and Moshelion (2017), plants may differ for abiotic stress tolerance, resilience or resistance. Tolerance is the ability of the plant to continue photosynthesis, under stress conditions. Resilience denotes the capability to recover and continue growth when moisture is present after drought. Resistance is the plant capability to withstand extreme stress that generally occur at the end of the growth cycle (terminal stresses), and to complete the growth cycle even if most of the leaves (green biomass) has been lost.

In field conditions, the great variability for heat and water stress types occurring on, together with strong genotype × environment interactions and dependence of phenotype on multiple quantitative traits, make complex the selection for improved agronomic performance (Dhanda et al., 2004). For this reason, since the 1970s (Pomeroy and Fowler, 1973), pre-breeding phenotyping under controlled environmental conditions has been commonly employed for functional characterization of varieties, progenies of crosses, mutants, etc. Nowadays, high-throughput non-destructive phenotyping technologies have greatly increased the number of experimental analyses of the wilting process (Humplík et al., 2015; Watt et al., 2020). Controlled environments provide greater reproducibility of experimental conditions and allow multiple stresses to be tested. However, for both non-automated and automated systems, either used in field or indoor, the developmental stage of the plants, stress history, spatial and temporal randomization of plants and micro-environmental fluctuations affect the phenotype which is scored (Yeh et al., 2012). Using separate pots to impose stress on plant, with different morphological-physiological traits, results in application of stresses which are not comparable in timing relative to development stages, and different intensity of the stress. Thus, those plants with greater leaf area, with thinner laminae and/or increased stomatal conductance, and well developed roots, will suffer onset of a water deficit more rapid and greater stress intensity, due to a greater velocity of water loss (Lawlor, 2012). Finally, the trait type to be measured by pre-field screening should be evaluated based on the required performance of plants in field. As an example, a tolerant plant that does not change its physiological activity under early drought and hence has no heavy green leaf loss, will have only a small reduction in yield. Analogously, resilient plants that show the ability to recover their functional activity soon after the stress has ended, are suitable for cultivation in environments where stress is short and intermittent. Highly resistant plants that survive and produce seeds also if with heavy loss of leaves, could have stable yield in cultivation environments where stress generally occurs at the end of the growing cycle (Negin and Moshelion, 2017).

By counting the number of dead (yellow) plants, together with yellow and green leaves, and hence traits that easily can also be detected by automatized phenotyping platforms, a physiological characterization of wheat, during progressive dehydration or after heat stress treatment were analyzed. Water deficit was applied by stopping irrigation, while in a separate experiment, four levels of heat stress were applied by increasing temperature up to 46°C. To minimize differences for stress response due to non-synchronous phenological development and to evaluate the capability of our experimental conditions to detect minimal phenotypic differences (like those that can be found between advanced breeding lines, at the final stages of a breeding program), 3 very genetically closed genotypes were used. They were “Trinakria” variety and 2 mutant lines of Trinakria. The first, called “Water-mutant”, has a high affinity for water fraction that is bound to the macromolecule surfaces (Rascio et al., 1999) and the second, named “Hg-mutant” is partially insensitive to $HgCl_2$, an aquaporin inhibitor. Both traits of the 2 mutants may have protective roles against dehydration stresses. Bound water is essential for structural integrity of bio-molecules (Vertucci and Leopold, 1987). Also, it may exert a passive control of osmotically active volume of the cell (Rascio et al., 2005). Aquaporins are membrane intrinsic proteins that facilitate water transport; their up-regulation or down-regulation under stress conditions is thought to be important for tolerance to drought stress (Sade et al., 2009).

MATERIALS AND METHODS

Thirty plastic pots (Figure 3) were each filled with 4 L of a mixture of soil and sand (50:50 v/v) with a maximum water-holding capacity of 0.32 g H_2O/g dry weight. The soil mixed to the sand was a clay-loam soil (Typic Chromoxerert), with the following physical and chemical characteristics: 36.9% clay, 50.5% silt, 12.5% sand, 15 mg/kg organic matter and pH 8. The pots were put in a 5 × 3.5 m^2 growth chamber, at 20°C/16°C, for a 10 h/14 h light/dark period. Plants were grown under 250-W high-pressure sodium lamps (Philips) and 400-W high pressure metal halide lamps (Philips). Radiation at the pot surface was 1000 µmol photons m^{-2} s^{-1} (400-700 nm) PAR. Fertilizer was applied before sowing: 18 g m^{-2} ammonium sulfate and mineral superphosphate. Cultivation of one plant per pot may create differences among them for stress history and dehydration velocity of soil, due to different growth of plants. For this reason, all pots were subdivided in three parts. Each of them represented one replicate and contained 24 seeds: 8 of “Trinakria”, 8 of “Water-mutant” and 8 of “Hg-mutant”. Seeds are part of the genotypic working collections, stored at CREA-CI of Foggia (Italy). Distribution of genotypes into each pot section and of pots within the growth chamber was random. After the emergence, to avoid damages to the roots, the plants were not thinned to the same number of plants per genotype, so the final number of plants examined for each per genotype and treatment (Table 1) was different. Before drought and after heat stress, the pots were always kept well-watered, with water loss restored every 2 days to about 80% of maximum soil capacity. When most of the plants had four fully-expanded leaves and hence were at the phase 13-14 of the Zadoks’ scale (Zadoks et al., 1974) 25 uniform pots, with 3-5

Table 1. Temperature cycles during drought stress, maximum temperature and duration of the four heat stress treatments and, number of pots (replicates) and total number of plants used per each genotype in each treatment.

Treatment		Temperature (°C)	Time (min)	Number of pots	No. of plants		
					Trinakria	Water-mutant	Hg-mutant
Control		20		4	13	21	20
Drought stress		20/16 (day/night)		4	19	24	26
Heat stress	Weak	44	60	5	24	24	26
	Moderate	44	165	4	17	20	20
	Strong	46	60	4	15	16	20
	Very strong	46	180	4	17	17	20

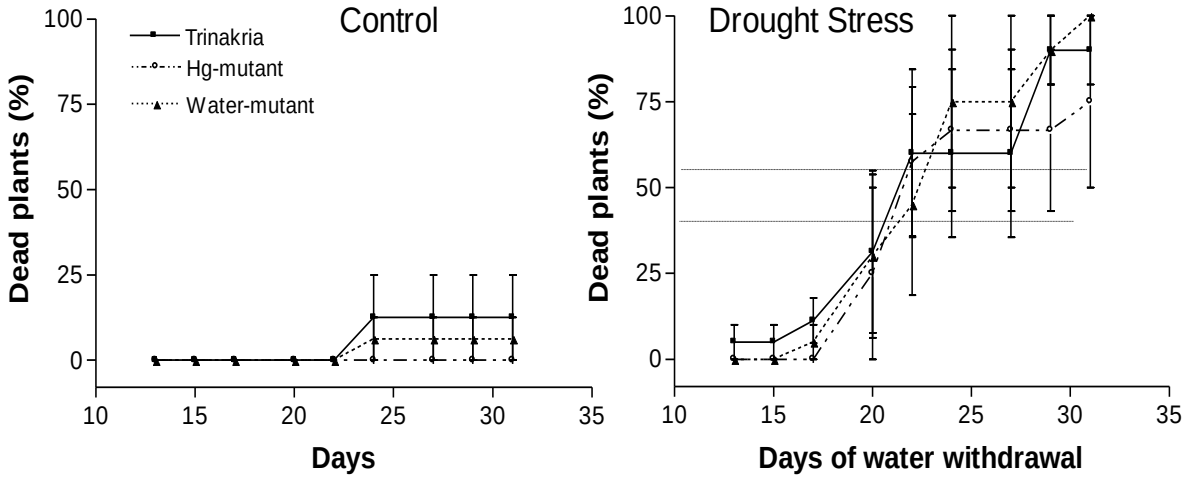

Figure 1. % changes for % of dead plants (for plant number before stress) of well watered (control) and droughted plants at increasing days from cessation of watering.

well growth plants per genotype and without signs of disease, were selected. Four pots were kept well watered at 20°C/16°C, for a 10 h/14 h light/dark period till the end of the experiment and used as controls.

Stress treatments

To perform heat stress experiments, a total of 21 well-watered pots were used. Four stress types, differing for temperature (44-46°C) and heat stress duration for a minimum of 60 to a maximum of 180 min were applied and for each of them 4 or 5 pots at a time, were used (Table 1). They were transferred in a thermostatic cabinet with radiation measured at pot surface equal to 60 µmol m^{-2} s^{-1} (400-700 PAR). Seven days after exposure to heat stress treatment, the number of died plants, green and yellow leaves per plant of each genotype in each pot (replicate) were counted.

For drought stress exposure, irrigation was interrupted on four pots. A total of 42 plants per genotype were used to dehydrate in the growth at 20°C/16°C, for a 10 h/14 h light/dark period. Twenty days after, when some symptoms of wilting were visible, the number of dead plants (totally yellow), the number of yellow and green leaves of surviving plants of each genotype in each pot (replicate), were counted. Leaves were classified as yellow if less than 60% of lamina was green. Then, the same measurements were repeated every 2-3 days, for 20 days.

Statistics

All results were analyzed using GraphPad Prism software, version 3.0. Differences among genotypes for percentage of died plants and green leaves were processed by one way analysis of variance (ANOVA), with variable number of replicates (pots) shown in Table 1. Means were compared by the multiple comparison test. Tukey regression analysis was performed to define any associations between the variables.

RESULTS

Water deficit stress

The % of dead plants per genotype, concerning the total plants counted before exposure to the drought stress imposition, as shown in Figure 1. Controls had the same percentage of living plants all over the experiment. On average, 50% of plants died about 22 days after water withdrawal (DAWW), while 75% of plants died between 24 and 34 DAWW (Water-mutant and Hg-mutant, respectively). However, there were no significant

Table 2. ANOVA analysis of the number of days required to kill 50% or 75% of plants, after the watering stop.

Variable	LD50%			LD75%		
	Trinakria	Water-mutant	Hg-mutant	Trinakria	Water-mutant	Hg-mutant
Mean	29.84	31.79	34.36	35.11	36.48	39.8
Std. Error	3.605	2.738	4.302	2.89	2.026	4.508
Coefficient of variation	24.17%	17.23%	25.04%	16.46%	11.11%	22.65%
F values (between genotypes)		0.3955^{ns}			0.5323^{ns}	
P		0.6845			0.6046	

Means of values extrapolated from linear regression of the percentage of dead plants vs day, of each replicate and genotype replicate, from 27 to 32 days from cessation of watering.

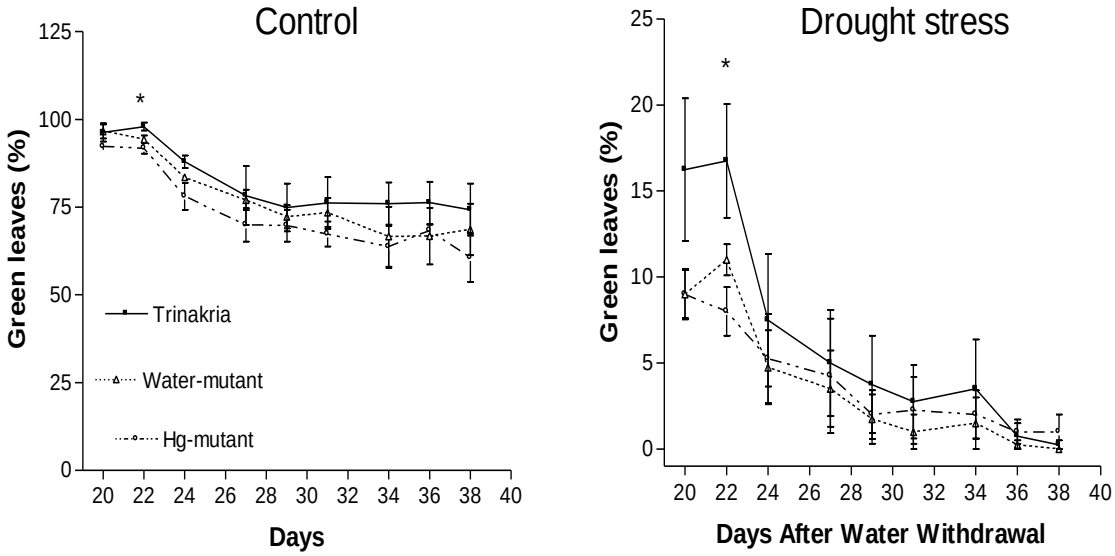

Figure 2. Genotypic comparisons for water stress tolerance estimated as changes with time % of green leaves (for total leaves of plants) of droughted plants and well watered plants (control). Means ± SE (n = 4).

differences between genotypes determined at increasing time intervals after water was withdrawn, on the basis of ANOVA (data not shown). Standard Error of the mean values of droughted plants increased with days of water withdrawal, because pot to pot differences in number of survived plants of each genotype were greater when drought stress intensity was stronger.

Using data collected from 13 to 32 days after water withdrawal, for each replicate of each genotype, the regression line was constructed and the number of days to have 50 and 75% of dead plants was interpolated. The estimated number of days required to kill 50% or 75% of plants did not differ significantly between genotypes (Table 2), based on ANOVA. At 22 DAWW (Figure 2) and hence under moderate drought stress, Trinakria cultivar had a significantly greater % of green leaves than the Hg-mutant (F=4.32; P=0.048), but with increased water deficit it showed a similar, decreasing trend to other genotypes and hence no significant differences were observed.

Heat stress

Figure 3A shows the plant's appearance before the heat treatment (44°C for 2 h, 45'), immediately after (Figure 3B) and 7 days after (Figure 3C). The number of dead plants was counted seven days after exposure to the stress. Based on Tukey's test (Figure 4), Trinakria mortality increased significantly compared to controls with moderate heat stress. For the other 2 genotypes, % mortality was significantly higher than controls after plant exposure to strong and very strong heat stress. None of the genotypes resisted to intense heat, because all plants died after strong or very strong heat stress (Figure 4). The % of green leaves, 7 days after stress relief is shown in Figure 5. Based on Tukeys' test, Trinakria had a significant reduction of green leaf number under very strong heat stress intensity, as compared to controls. In contrast, after exposure to weak and moderate heat stress, the % of green leaves of both Water-mutant and Hg-mutant was lower than control.

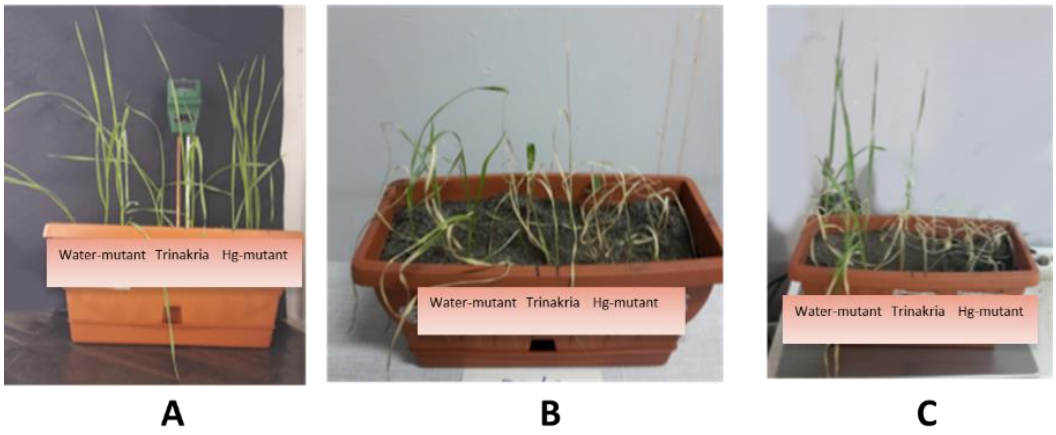

Figure 3. Representative pots showing the three genotypes before the exposure exureto heat stress treatments (A), immediately after plant exposure to 44 °C for 2h, 45' (B) and 7 days after (C).

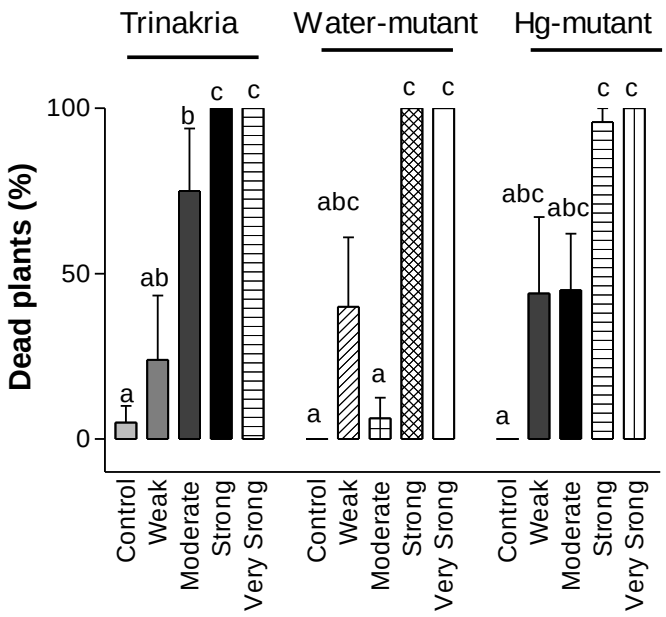

Figure 4. % of dead plants days after exposing or not (control) 3 wheat genotypes to different heat stress treatments. Means (n=4-5) and SE are shown. Within genotypes, bars sharing different letters are significantly different (P<0,05) according to Tukey's HSD test.

DISCUSSION

In temperate climates, water deficit or high-temperature stresses that occur at vegetative stages are often intermittent and of low intensity, but they greatly affect crop yield. To save yield, late drought stress needs resistant plants that survive and produce seeds even if they completely lose the leaves. Tolerant and/or resilient varieties that under abiotic stress are photosynthetically active and hence do not lose their green biomass, could be better useful under early stress.

Results showed that in the system employed, water deprivation caused the death of about 50% of plants 22 days after withdrawing water. Days to kill 75% of plants, ranged from 24 days for Water-mutant, to 32 days for Hg-mutant, but this trait and days to kill 50% of plants did not differ significantly between genotypes. The magnitude of genetic components of variance is, generally, lower under stress conditions than under control conditions (Dhanda et al., 2004). Moreover, the drastic treatment necessary

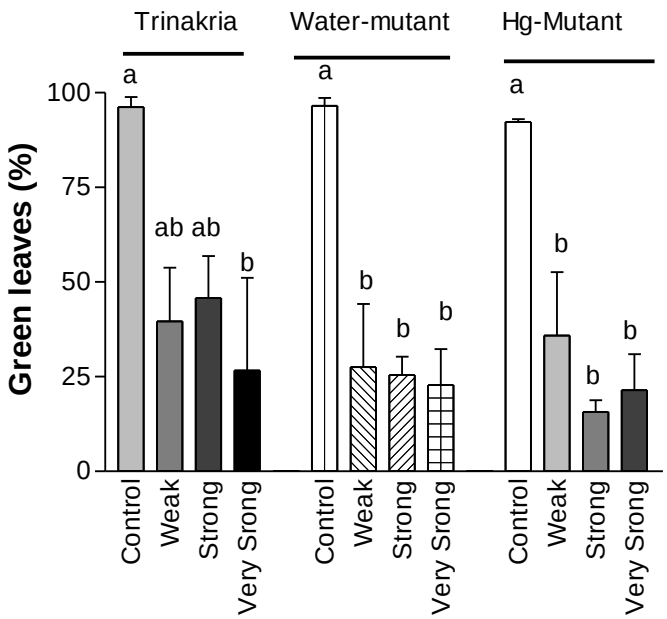

Figure 5. % of green leaves with respect to the starting number of leaves per plants, calculated at seven days after exposing or not (control) 3 wheat genotypes to different heat stress treatments. Means (n=4-5) and SE are shown. Bars with different letters are significantly different (P<0.05) according to Tukey's HSD test.

to kill plants increased the well-known variability of plant growth existing within controlled-environment chambers (Measures et al., 1973; Massonnet et al., 2010; Porter et al., 2015). Other authors (Sallam et al., 2018), by using higher average day-night temperature, a single genotype per pot and smaller pots had an average time to 50% wheat wilting, about 13 days shorter than that here. They also observed significant genotypic differences, within a *ril* population, derived from crosses of more genetically distant parents, as compared to wild type and mutant lines probably due to greater genotypic differences within the *ril* population; lower duration of the cultivation phase necessary to kill 50%; inability to impose the same speed of dehydration on separate pots if they contain genotypes with different morpho-physiological characteristics, already at the beginning of exposure to the stress.

In contrast to what observed for drought response, protective mechanisms that allow plant acquisition or loss of thermotolerance exist and they are under both genetic and epigenetic control (Larkindale et al., 2005; Liu et al., 2015). In this work, too genotypic differences were observed for heat stress effects on plant mortality, because Trinakria cv. performance was significantly worse than that of the mutants. Starting from moderate stress, its mortality increased compared to controls, while for the other 2 genotypes % mortality increased significantly only after the exposure to strong or very strong heat stress. Furthermore, some methodological factors could be the basis for the minor differences in the genotypic response to water stress compared to heat stress. Water withdrawal lasted twenty days. In this relatively long period, changes in the micro-environmental conditions occurred within the growth chamber which differentially modified the stress history of each pot and hence plant growth. The consequences were that, under drought conditions, large plant to plant differences were observed within replicates of the same genotype. On the contrary, thermal stress exposure lasted a few hours, after which all the surviving plants could express their recovery potentiality because optimal conditions were ensured to all plants.

Trinakria cv. Appeared to preserve better the photosynthesizing apparatus because, one week after the withdrawn of watering under weak or moderate stress, it had the same % of green leaves. In contrast, the 2 mutants had 50% fewer green leaves. The general lack of differences in genotype performance under strong high temperature stress, suggests that physiological mechanisms that differentiate the genotypes are unable to affect their performance at temperatures higher than 45°C.

Conclusions

This study was performed to evaluate the genotypic plant

performance under abiotic stress by counting dead (yellow) plant, together with yellow and green leaves per plants of Trinakria cv., Water-mutant and Hg-mutant. Significant differences among genotypes for the examined traits have been observed. This result suggests that the used method was effective in showing differential plant performances plants under abiotic stress conditions, but further experiments in field are necessary to test the agronomic performance of the same genotypes after exposure to early stress. At the same time, to apply this approach in breeding programs by using automatized systems, pots will have to be designed for simultaneous sowing and screening of many genotypes, which will provide equal conditions of competitiveness of root systems and speed of soil dehydration. Because mutations have functional consequences in terms of abiotic stress response, these mutants are potential sources of traits to be used in traditional breeding programs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Evaluation of plant extracts for the management of Cercospora leaf spot of groundnut (*Arachis hypogaea* L.)

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Groundnut (*Arachis hypogaea* L.) is a leguminous crop with high economic and nutritional value. However, increased production is hampered by Cercospora leaf spot (CLS) caused by *Cercospora arachidicola* and *Cercosporidium personatum*. Studies were conducted *in vitro* and *in vivo* to evaluate the efficacy of aqueous extracts of desert date seed (DDSE), neem seed (NSE), jatropha seed (JSE) and tobacco leaf (TLE) for the management of CLS. The antifungal activities of 25, 50, 75 and 100 g/l concentrations of each of the plant extracts was assessed *in vitro* on potato dextrose agar using the food poison technique. The field study was a factorial experiment consisting of 18 treatments laid in a Randomised Complete Block Design with four replications over two cropping seasons. The *in vitro* results revealed that all the botanicals at 100 g/l recorded the highest inhibition percentages. DDSE at 100 g/l significantly ($P < 0.001$) inhibited the highest mycelia growths compared to other levels of plant extracts used with inhibition percentages of 90.33 and 84.96% in *C. arachidicola* and *C. personatum*, respectively. Three out of the four aqueous extracts (DDSE, NSE and JSE) at 100 g/l significantly ($P < 0.05$) lowered disease incidence, severity and defoliation in the field and increased yield. Pod yield was significantly ($P < 0.05$) higher in plants treated with JSE, NSE, DDSE and Topsin-M, compared to those treated with TLE and the negative control plants. For most of the parameters, DDSE produced similar results as Topsin-M followed by NSE and JSE. Farmers can adopt DDSE, NSE and JSE as alternatives to fungicides leading to minimal effect on the environment since they are biodegradable.

Key words: Cercospora leaf spot, plant extracts, groundnut, incidence, severity, aqueous.

INTRODUCTION

Ghana is a major producer of groundnuts (*Arachis hypogaea* L.) in West Africa with nearly all production coming from northern Ghana (DAI and Nathan Associates, 2014). Despite its economic importance in the northern parts of Ghana, its current average yield of 0.8 t/ha is not up to its potential yield of 2.5 to 3.0 t/ha

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(Kombiok et al., 2012; Tanzubil et al., 2017). This large yield gap is attributable to diversity of production constraints, notably pests and diseases, low inherent yielding varieties, low and high temperatures at certain growth stages of the crop, non-irrigated cultures and increased cultivation on marginal lands among others (Ambang et al., 2011; Tshilenge-Lukanda et al., 2012). Nonetheless, Cercospora leaf spot (CLS) caused by *Cercospora arachidicola* and *Cercosporidium personatum* is the most destructive foliar disease in West Africa (Mohammed et al., 2019).

Control of CLS with fungicides is effective but it largely depends on inorganic fungicide applications which are too expensive for indigenous farmers in Northern Ghana (Nutsugah et al., 2007; Akinbode, 2010; Jordan et al., 2012). Aside from this, chemical control also raises environmental and health concerns (Jordan et al., 2012). In Ghana, Imoro et al. (2019) reported that mode of storage of pesticides by farmers have adverse effects on their health as well as the environment.

Although fungicides are effective for controlling the disease, awareness about environmental pollution caused by misuse of fungicide, tolerant pathogens strains, non-availability of both fungicides and their application technology to resource-limited farmers, have necessitated the use of more economical and ecologically-friendly alternatives. There are reports on the potential of some plants with fungicidal properties which can be used for controlling diseases. For instance, Sowley et al. (2017) reported that *Azadirachta indica* seed and *Cassia alata* leaf extracts controlled seed borne fungi of maize. The study sought to determine the efficacy of some botanicals for the management of Cercospora leaf spot of groundnut.

MATERIALS AND METHODS

Experimental site

Laboratory studies were carried out in the Spanish laboratory at the University for Development Studies, Nyankpala campus, during 2014 and 2015 cropping seasons whilst the field studies was conducted under rain-fed conditions in 2014 and repeated in 2015 on the experimental field of the Faculty of Agriculture, University for Development Studies, Nyankpala campus.

Sample collection

A. indica and *Jatropha curcas* seeds, as well as *Nicotiana tabacum* leaves, were collected from Fooshegu and Tamale whilst *Balanites aegyptiaca* seeds were obtained from Jantong-Dashee in the East Gonja district. The plant materials were obtained from healthy plants. The seed and leaf samples were stored in polyethene bags until required.

Optimization of plant extract concentrations

The various plant materials (that is, neem, *J. curcas*, desert date seeds and tobacco leaves) were collected, washed with several changes of sterile distilled water, and air-dried to constant weight for 10 days; tobacco leaves were cut into tiny pieces before washing and drying. For seeds, the coats were removed before pounding. The dried plant materials were pounded separately with sterile mortar and pestle and sieved with a fine sterile cheesecloth to obtain a fine powder. The powders obtained were sieved through a screen with a mesh size of 0.4 mm to obtain a fine powder. Cold aqueous extracts of the samples were prepared separately by adding 25, 50, 75 and 100 g of the powder samples into conical flasks. Each sample was wrapped in cheesecloth and soaked in 1 L of water for 24 h. The cloth was squeezed and the extract was filtered. 2 g of an emulsifier ('key soap') was added to each filtrate to facilitate sticking. Based on the results of the *in vitro* studies, 100 g/l was identified as the most effective concentration of the extract and used for the field study.

Phytochemicals screening of the plant extracts

Alkaloids, saponins, tannins, steroids and terpenoids were detected with the methods described by various workers.

Following the methods of Edeoga and Okwu (2005) and Kareru et al. (2008), the presence of alkaloids were detected in the plant extracts. The methods described by Wall et al. (1954) and Kareru et al. (2008) were used for testing for saponins. The methods described by Sabri et al. (2012) were also used for detecting tannins and phenolic compounds. Similarly, Salkowski test was also used for the detection of steroids and terpenoids.

Isolation and identification of *C. arachidicola* and *C. personatum*

Potato Dextrose Agar (PDA) was prepared based on the manufacturer's recommendation of 39 g/l. The media was autoclaved at a temperature of 121°C and a pressure of 1.02 kg/cm^3 for 15 min. It was then amended with 1 g of chloramphenicol before dispensing into sterile Petri dishes and allowed to cool. Pieces of infected groundnut leaves were sterilised with 4% sodium hypochlorite. The sterile pieces of leaf were placed on the PDA plates at equidistant points and kept in a freezer at a temperature of 28°C for 48 h. Following the procedure of Barnett and Hunter (1998), fungi were identified based on morphological and cultural features. Slides of pure cultures obtained were prepared and observed under a compound microscope (Celestron LCD Digital microscope, Model number 44340, UK).

Determination of the inhibitory effect of the aqueous plant extracts on mycelia growth of *C. arachidicola* and *C. personatum*

Food poison technique was used for the infected samples of the three groundnut cultivars ('Chinese,' Mani-pintar and 'Bugla'). Five millilitres of each extract concentration (that is, 25, 50, 75 and 100 g/l) of the supernatant of the test extracts were dispersed in 20 ml potato dextrose medium in 90 cm Petri dishes, swirled to blend and allowed to solidify. A 5 mm disc of five days old culture of the two test fungi each was inoculated separately at the centre of the PDA medium and incubated at 28 ± 2°C. The growth of each fungus diametrically was taken for 7 days on daily basis. For positive controls, 5 ml of Topsin-M prepared at the recommended rate (1 g/l) as well as 2 and 3 g/l were used for the amendment. The negative controls had only the PDA medium without the extracts. The colony diameter representing mycelia growth was measured using a transparent rule on a daily basis after inoculation for seven days.

The percentage inhibition of mycelial growth was calculated as follows (Begum et al., 2010):

I=(C-T/C) ×100

where I = Percentage inhibition, C = Radial growth in control, T =Radial growth in treatment.

Pathogenicity test of *C. arachidicola* and *C. personatum*

The seedlings of the 'Chinese' cultivar were raised on loamy soil contained in perforated black polythene bags (15 × 30 cm^2) in a plant house with an average temperature of 28°C. Twenty-one-day old plants were pinpricked and sprayed with a suspension containing mycelia of *C. arachidicola* and *C. personatum* [1 × 10^3 cfu mL^{-1}] prepared in sterile distilled water, except the control plants. Pathogenicity test of the fungal isolates was based on the method of Eman (2011).

Measurement of disease parameters

Disease incidence

Five plants were randomly selected and tagged for disease assessment in each plot per treatment during 2014 and 2015 cropping seasons. Disease incidence was recorded on these five plants in each plot for every treatment before treatment application. Mean % incidence was calculated with the formula (Chaube and Pundhir, 2009):

$$\text{Disease Incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Disease severity and disease severity index (%)

Five plants in each plot per treatment were randomly selected and tagged. These plants were used to assess the severity of CLS using the Florida scale system of 1 - 10, where 1 = no leaf spot and 10 = plants completely defoliated and killed by leaf spots (Chiteka et al., 1988). The descriptive keys were used to determine the severity of the disease.

Disease severity index (DSI) was then calculated using the equation proposed by Kobriger and Hagedorn (1983):

$$\text{DSI} = \sum \frac{(\text{severity} * \text{number of plants in the class}) * 100}{(\text{Total number of plants rated}) * (\text{Number of class} - 1)}$$

The evaluation of early and late symptoms of CLS was done after every 14 days starting from the 3rd WAP.

Yield and yield parameters

Yield characteristics such as the weights of 100 pods and 100 seeds from each plot per treatment were randomly picked and weighed using a Sartorious scale balance. The average weight of five counts was then taken as the weight of 100 pods and 100 seeds for each plot per treatment. Similarly, the total dry pod and seed yields of groundnut from the respective treatments were determined using the four median rows in each plot per treatment. The weights of groundnuts harvested from each plot were extrapolated to total pod yield per hectare basis.

Experimental design

The field experiment was a 6 × 3 factorial laid out in a Randomised Complete Block Design (RCBD) with four replications per treatment. Each replication consisted of 18 experimental plots measuring 4 × 5 m^2. The factor levels comprised three groundnut cultivars, namely: Chinese, Mani-Pinta and Bugla, and four plant extracts (desert date seed, neem seed, jatropha seed and tobacco leaf) with Topsin-M and water as positive and negative controls, respectively, producing 18 treatments. All groundnut cultivars (Chinese, Mani-pintar and Bugla) were obtained from the Seed Unit of the Savannah Agricultural Research Institute (SARI, 2014).

One seed each of the groundnut was sown per hole at a depth of about 5 cm in a planting distance of 50 cm × 20 cm. Each plot consisted of 10 rows and four median rows which were used for disease assessment and yield records. Treatments were applied every 2 weeks from 2 to 13 weeks after planting (WAP) using a 15-L knapsack sprayer.

The treatments used were as follows: Neem seed extract (NSE) + Chinese, Neem seed extract (NSE) + Mani-Pintar, Neem seed extract (NSE) + Bugla, Desert date seed extract (DDSE) + Chinese, Desert date seed extract (DDSE) + Mani-Pintar, Desert date seed extract (DDSE) + Bugla, Tobacco leaf extract (TLE) + Chinese, Tobacco leaf extract (TLE) + Mani-Pintar, Tobacco leaf extract (TLE) + Bugla, Jatropha seed extract (JSE) + Chinese, Jatropha seed extract (JSE) + Mani-Pintar, Jatropha seed extract (JSE) + Bugla, Topsin-M + Chinese, Topsin-M + Mani-Pintar, Topsin-M + Bugla, Water + Chinese, Water + Mani-Pintar and Water + Bugla.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using Genstat Discovery (12th Edition). Treatment means were separated using the Least Significance Difference (LSD) at 5% significant level.

RESULTS

Phytochemical composition of plant extracts

Neem seed and tobacco leaf extract treated plants had the highest number of phytochemicals while jatropha seed extract had the lowest (Table 1). All the extracts contained alkaloids, tannins and phenolic compounds. Only desert date seed, neem seed and tobacco leaf contained saponins. Steroids were present in only neem seed and terpenoids in only neem seed and tobacco leaf.

Isolation of causative organism

The fungal pathogens *C. arachidcola* and *C. personatum* were isolated from infected leaves of three groundnut cultivars Bugla, Mani-Pinta and Chinese and confirmed as the causative agents of Cercospora leaf spot diseases of groundnut. The conidium of *C. arachidicola* is sub hyaline or pale yellow, obclavate or cylindrical and septate with rounded base and sub-acute tip (Figure 1A). However, in the case of *C. personatum* conidium was obclavate or cylindrical and light coloured. The base is shortly tapered with a conscipicous hilum (Figure 1B).

Table 1. Phytochemical constituents of plant extracts.

Phytochemical constituent	Jatropha seed	Desert date seed	Neem seed	Tobacco leaf
Alkaloids	+	+	+	+
Saponins	-	+	+	+
Tannins and phenolic	+	+	+	+
Steroids	-	-	+	-
Terpenoids	-	-	+	+

+ = Present; - = Absent.

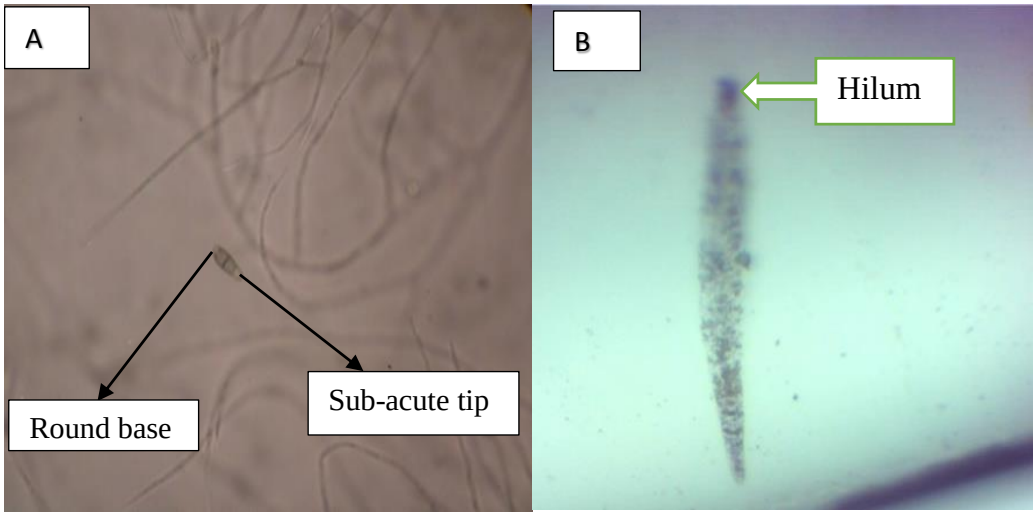

Figure 1. Conidium of *Cercospora arachidicola* (A) and broken conidium of *Cercosporidium personatum* (B) with distinct hilum at base.

Growth inhibition of fungal isolates

Topsin-M treated plants produces 100% mycelia growth inhibition (Table 2). All aqueous extract at 100 g/l recorded the highest inhibition percentages. Desert date seed extract (DDSE) at 100 g/l significantly ($P < 0.001$) inhibited the radial growths of both fungi compared to all levels of concentrations of plant extracts used with inhibition percentages of 90.33 and 84.96% in *C. arachidicola* and *C. personatum*, respectively. Even aqueous DDSE at 75 g/l was comparable to neem seed extract (NSE) at 100 g/l but was significantly higher ($P < 0.001$) than 100 g/l of jatropha seed extract (JSE) and tobacco leaf extract (TLE). Apart from DDSE at 100 and 75 g/l, NSE 100 g/l was the next best with percentage mycelia inhibition of 80.88 and 72.32% in both *C. arachidicola* and *C. personatum*, respectively. Different concentrations of tobacco leaf extract at 25, 50, 75 and 100 g/l reduced mycelial growth of both fungi. However, TLE was not as effective compared to DDSE, NSE and JSE in fungi-toxic activity against Cercospora leaf spot diseases (Table 2).

Disease incidence

In both 2014 and 2015 cropping seasons, plants treated with desert date extract (DDSE) recorded the lowest disease incidence with almost the same effect as Topsin-M the positive control from 3 to 7 weeks after planting (Figure 2). Tobacco leaf extract (TLE) recorded the highest. The disease incidence for all the plant extract treatments was generally lower in 2015 compared to 2014. For instance, by 7 WAP in 2014, Neem leaf seed extract (NSE) treated plants had recorded about 50% disease incidence compared to 20% disease incidence during the same period in 2015. By 7 WAP in both seasons, TLE treated plants and those which were treated with neither plant extracts nor fungicide, recorded 100% disease incidence.

Disease severity index

In the field experiment, both early leaf spot (ELS) and late leaf spot (LLS) were more severe in all treatments during 2015 cropping season (Table 3). In 2014 and 2015

Table 2. Effects of plant extracts on mycelia growth of the fungi.

Treatment	Growth inhibition (%)	
	C. arachidicola	*C. personatum*
Topsin-M (1 g/L)	100.00[a]	100.00[a]
Topsin-M (2 g/L)	100.00[a]	100.00[a]
Tops-M (3 g/L)	100.00[a]	100.00[a]
DDSE (25 g/L)	73.43[ef]	71.61[de]
DDSE (50 g/L)	77.94[de]	75.06[cd]
DDSE (75 g/L)	82.16[cd]	78.30[c]
DDSE (100 g/L)	90.33[b]	84.96[b]
JSE (25 g/L)	56.88[ij]	49.92[i]
JSE (50 g/L)	60.56[hi]	59.47[gh]
JSE (75 g/L)	68.71[fg]	62.91[g]
JSE (100 g/L)	75.66[ef]	67.28[ef]
NSE (25 g/L)	58.47[i]	60.20[g]
NSE (50 g/L)	64.35[gh]	64.63[fg]
NSE (75 g/L)	70.15[fg]	70.65[def]
NSE (100 g/L)	80.88[c]	73.32[cd]
TLE (25 g/L)	49.34[l]	54.01[hi]
TLE (50 g/L)	50.57[kl]	56.46[hi]
TLE (75 g/L)	51.53[kl]	57.59[h]
TLE (100 g/L)	54.50[jkl]	59.38[gh]
Control (Water)	0.00	0.00
Fr (*P*)	<0.001	<0.001
LSD (0.05)	6.461	6.583

Means with different letters within the same column are significantly different at 5%. Neem seed extract (NSE), Desert dates seed extract (DDSE), Jatropha seed extract (JSE) and Tobacco leaf extract (TLE).

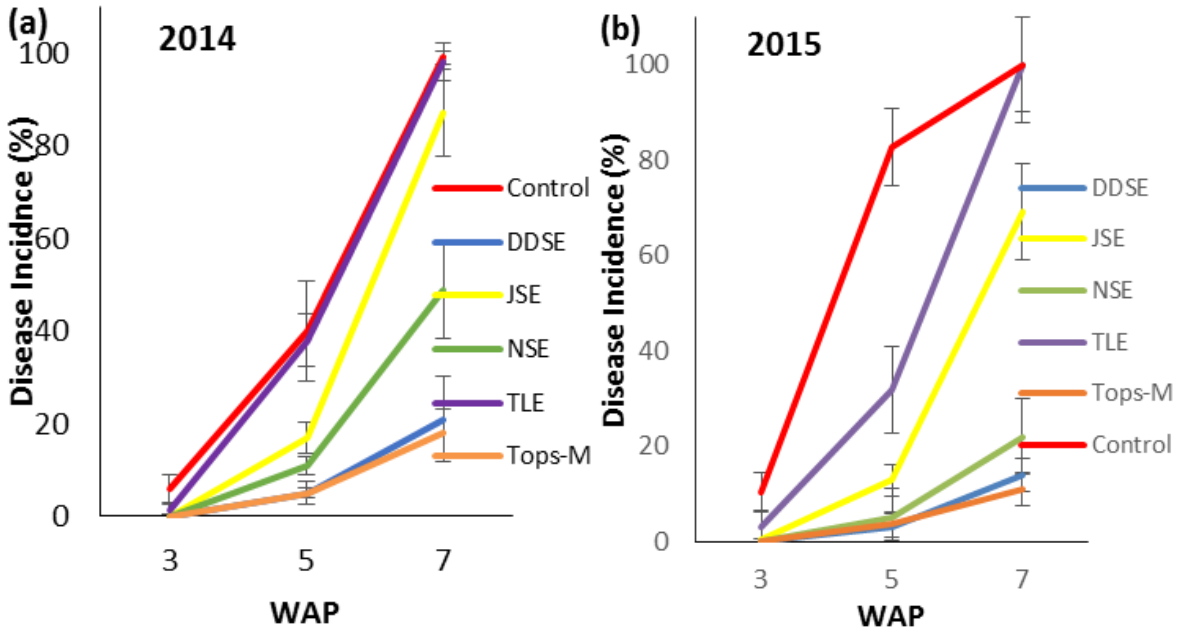

Figure 2. Influence of some botanicals on disease incidence of CLS of groundnut in 2014 and 2015 cropping seasons. Neem seed extract (NSE), Desert Date seed extract (DDSE), Jatropha seed extract (JSE), and Tobacco leaf extract (TLE).

Table 3. Effects of plant extracts on disease severity on three cultivars of groundnut in 2014 and 2015 cropping seasons.

Treatment		Disease severity index (%) cropping seasons			
		Early leaf spot (ELS)		Late leaf spot (LLS)	
Plant extract	**Cultivars**	**2014**	**2015**	**2014**	**2015**
DDSE	Mani-Pinta	22.00^{ab}	23.08^{a}	20.42^{a}	21.42^{ab}
	Bugla	21.42^{a}	22.75^{a}	21.08^{ab}	21.67^{ab}
	Chinese	21.75^{ab}	23.5^{a}	20.00^{a}	21.75^{ab}
JSE	Mani-Pinta	26.5^{abcd}	29.92^{abc}	26.08^{abc}	29.42^{abcd}
	Bugla	25.08^{abcd}	28.08^{ab}	27^{abcd}	29.75^{abcd}
	Chinese	28.5^{bcde}	32.33^{bcd}	26.50^{abc}	30.83^{bcde}
NSE	Mani-Pinta	23.42^{abc}	26.92^{ab}	24.17^{ab}	26.00^{abc}
	Bugla	23.08^{abc}	25.83^{ab}	24.42^{ab}	25.58^{abc}
	Chinese	25.42^{abcd}	29.17^{abc}	24.00^{ab}	27.00^{abc}
TLE	Mani-Pinta	29.58^{cde}	36.33^{cde}	28.17^{abcd}	35.25^{cde}
	Bugla	28.75^{bcde}	32.58^{bcd}	28.33^{abcd}	33.83^{cde}
	Chinese	36.08^{ef}	40.58^{ef}	30.58^{bcd}	38.75^{def}
Topsin-M (positive control)	Mani-Pinta	19.92^{a}	22.83^{a}	19.25^{a}	20.67^{a}
	Bugla	20.33^{a}	22.33^{a}	19.00^{a}	20.33^{a}
	Chinese	21.83^{ab}	24.00^{a}	19.67^{a}	21.00^{ab}
Water (negative control)	Mani-Pinta	30.50^{de}	39.17^{de}	35.08^{de}	39.83^{ef}
	Bugla	29.08^{cde}	35.92^{cde}	33.42^{cde}	37.17^{def}
	Chinese	39.83^{f}	47.28^{f}	42.58^{e}	47.92^{f}
Fr (*P*)		<0.001	<0.001	<0.001	<0.001
LSD (0.05)		7.001	7.754	9.920	10.379

cropping seasons, plants of the three cultivars (Bugla, Chinese and Mani-Pinta) treated with DDSE recorded a significantly lower ($P < 0.001$) severity similar to Topsin-M, whereas those treated with TLE recorded significantly higher ($P < 0.001$) severity comparable to the negative control. A similar trend was observed for the late leaf spot in both seasons

Yield and yield parameters

Plants treated with DDSE in both cropping seasons recorded significantly higher ($P < 0.001$) pod yield while those treated with TLE recorded the lowest (Table 4). However, the pod yield of DDSE treated plants in 2015 (1275 kg/ha) was higher than that in 2014 (931 kg/ha). Significant differences ($P < 0.001$) were observed among the treatments in both seasons except jatropha seed extract (JSE) and neem seed extract which yielded 931 and 1004 kg/ha, respectively but the differences were not significant.

Generally, plants treated with DDSE in both seasons produced heavier seeds than all the other treatments except Topsin-M the positive control (Table 4). Dry seed yield from all treatments in 2015 were higher than those produced in 2014. For instance, seed yield from DDSE treated plants in 2014 and 2015 were 992 and 751 kg/ha, respectively.

In both cropping seasons, DDSE treated plants produced a significantly higher 100 pod weight than all the other treatments except Topsin-M. Plants treated with TLE recorded the least 100 pod weight in both seasons (Table 4).

In 2014 cropping season plants treated with DDSE produced a higher 100 seed weight that all the other treatments but the differences were not significant at 5%. However, in 2015 DDSE treated plants recorded 100 seed weight of 49.82 g which was comparable to that of Topsin-M treated plants (50.72) but significantly higher ($P < 0.001$) than the other treatments (Table 4).

DISCUSSION

Alkaloids, tannins and phenolic compounds were found in all the botanicals used. This confirms the report that plant

Table 4. Effects of plant extracts on 100 pod weight, 100 seed weight, dry pod and seed yields in 2014 and 2015 cropping seasons.

Plant extract	Dry pod yield (kg/ha)		Dry seed yield (kg/ha)		100 pod weight (g)		100 seed weight (g)	
	2014	2015	2014	2015	2014	2015	2014	2015
Desert Date Seed Extracts	931.00^{b}	1275.00^{b}	751.00^{b}	992.00^{a}	87.90^{ab}	87.57^{a}	39.50^{b}	49.82^{a}
Jatropha seed extract	729.00^{c}	931.00^{c}	546.00^{c}	698.00^{b}	75.40^{cd}	56.39^{c}	36.70^{b}	32.86^{c}
Neem Seed Extract	875.00^{b}	1004.00^{c}	688.00^{b}	786.00^{b}	85.30^{bc}	67.07^{b}	37.50^{b}	37.31^{b}
Tobacco leaf Extract	626.00^{c}	692.00^{d}	504.00^{c}	570.00^{c}	74.50^{d}	49.86^{d}	37.20^{b}	30.19^{d}
Topsin-M (positive control)	1095.00^{a}	1322.00^{a}	922.00^{a}	1045.00^{a}	96.80^{a}	88.23^{a}	46.70^{a}	50.72^{a}
Water (negative control)	426.00^{d}	581.00^{d}	306.00^{d}	430.00^{d}	45.70^{e}	49.86^{d}	23.60^{c}	27.67^{d}
Fr (*P*)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD (0.05)	103.6	140.9	80.3	124.9	10.46	5.397	5.21	3.75

extracts contain phytochemicals such as phloretin, tannins, allicins, and azadirachtin which have antimicrobial properties (Gurjar et al., 2012). Desert date seeds, neem seeds and tobacco leaves contained saponins. Terpenoids were detected in neem seeds and tobacco leaves. Neem seeds also contained steroids. Kishore et al. (2001) observed the manifestation of these bioactive compounds in different plant materials. It has been noted that plant extracts with antimicrobial property can be either specific or broad spectrum in action against pathogens (Gurjar et al., 2012).

The fungal pathogens isolated and identified from infected groundnut leaves were *C. arachidcola* and *C. personatum* which are the causative agents of Cercospora leaf spot diseases of groundnut. The conidium of *C. arachidicola* was sub-hyaline or pale yellow, obclavate or cylindrical and septate with a rounded base and sub-acute tip. McDonald et al. (1985) observed related morphological characteristics. However, the conidium of *C. personatum* was obclavate or cylindrical, light coloured and the base was shortly tapered with a conspicuous hilum. This morphological description is similar to that reported by Ijaz (2011).

The *in vitro* studies showed significant differences ($P > 0.001$) among plants treated with various botanicals and the control treatment. The results also indicated that the efficacy of the different extracts is also dependent on the type of plant material. Therefore, the level of inhibitions of *C. arachidicola* and *C. personatum* were dependent on the type of plant extract and concentration level. This conforms to the works of Ibiam and Nwalobu (2016) who postulated that aqueous extract of *Asipilia africana* and *Vernonia amygdalina* decreased the vegetative growth of *Hendersonia celtifolia* as concentration increases. All extracts at 100 g/l especially desert date seed, neem seed and jatropha seed extract significantly inhibited the vegetative growth of the test fungi compared to tobacco leaf extract and control (negative). Again, this confirms the findings of Akinbode (2010) who observed that some botanicals at 100% concentration significantly inhibited the growth of *Curvularia lunata*. However, TLE was not as effective compared to DDSE, NSE and JSE in its fungi-toxic activity against Cercospora leaf spot diseases.

The results showed that plant extracts lowered the disease severity index with desert date seed extract at 100 g/l recording the least severity index percentage which was statistically similar to Topsin-M at 2 g/l. Plants treated with 100 g/l each of DDSE, NSE, JSE and TLE produced heavier pods. This can be attributed to the phytochemicals since some of them are known to induce growth. This supports the work of Ambang et al. (2011) that an increase in the concentration of *Thevetia peruviana* seed extract reduced the rate of spread of Cercospora leaf spot of groundnut.

Groundnut plants of all the three cultivars when sprayed with aqueous desert date seed extract had consistently lower disease incidence and severity in both 2014 and 2015 cropping seasons and the effect was comparable to the positive control (Topsin-M). This was followed by neem seed extract and then jatropha seed extract with tobacco leaf extract being the least. Therefore, the efficacy of the plant extracts could be attributed to the presence of the fungitoxic phytochemicals such as phenolic compounds, steroids and terpenoids. This confirms that phenols and saponins extracted from higher plants possess anti-fungal compounds against various microbes (Halama and Haluwin, 2004). However, the difference in efficacy of the four plant extracts could be attributed to the differences in the nature of their active ingredient (Ngegba et al., 2017). DDSE, NSE, JSE and TLE significantly increased yield parameters including 100 pod weight, 100 seed weight, dry pod and seed yields in both 2014 and 2015 cropping seasons compared to the negative control. This could be attributed to the antifungal properties which retarded or inhibited the activity of the fungi leading to a decrease in disease incidence and disease severity. This could have led to an increase in photosynthetic activity which enhanced vegetative growth, net assimilation and dry matter accumulation, resulting in more yield. The findings of this study support the report by Hossain and Hossain (2013) that plant extracts maximize yield of groundnut

comparative to the control (negative).

Conclusion

Desert date seed, neem seed, jatropha seed and tobacco leaf extracts suppressed the growth of *C. arachidicol*a and *C. personatum*. The studies showed that efficacy increases as concentrations of plants extracts increases and the level of efficacy also depends on the type of plant material used. All concentrations at 100 g/l extracts significantly inhibited the vegetative growth of the test fungi. The use of desert date seed extract (DDSE), neem seed extract (NSE) and jatropha seed extract (JSE) consistently reduced disease incidence and severity of both *C. arachidicola* and *C. personatum* than tobacco leaf extract (TLE) and negative control. However, the most effective plant extract was aqueous DDSE which was nearly as potent as the positive control, Topsin-M in 2014 and 2015 cropping seasons followed by NSE and JSE. Since DDSE was the most effective in both *in vitro* and field studies, it is recommended for the management of Cercospora disease of groundnut by farmers as an alternative to expensive inorganic fungicides.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Ecology and morphological characterization of the genus Phellinus sensu-lato (Basidiomycetes, Hymenochaetaceae) in Burkina Faso

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Phellinus **sensu lato is a genus of polypores that are morphologically, biologically and phylogenetically highly diverse. This genus is composed of sessile and lignicolous species. Species belonging to this genus are found in all regions of the world where they decompose wood or live as tree parasites. In Burkina Faso, few studies have been conducted on this taxonomic group. Thus, these collections were carried out respectively in the classified forest of Kou (Bobo Dioulasso) and the Tin landscape (Orodara). These two sites have forest formations that provide a biotope favourable to the development of polypores. Data collection was carried out randomly along the 200 to 300 meter long transects. Basidiomes were collected from the trunk, branches or roots of forest trees using a machete. The geographic coordinates as well as the morphological characteristics of each sample were carefully noted in the field. Anatomo-morphological and ecological studies permitted to identify three (03) species. They are *Phellinus cf. igniarius, Phellinus* cf*. leavigatus and Phellinus* cf**. robustus.** All these species are perennial, tough and have a woody consistency. These species were collected for the first time on *Parkia biglobosa*, and *Anogeissus leiocarpus* in Burkina Faso.**

Key words: *Phellinus*, polypores, ecology, morphology, Burkina Faso.

INTRODUCTION

Hymenochaetaceae constitute a family of polypores belonging to the order Hymenochaetales. This group is highly diverse morphologically, biologically and phylogenetically (Ryvarden, 1991; Hibbett and Thorn, 2001). Species of the Hymenochaetaceae family arefungi responsible for white rot in wood ; they are either saprotrophs, facultative or obligate parasites of trees and most taxa are of tropical distribution (Hawksworth et al., 1995; Ryvarden, 1991; Ryvarden and Gilbertson, 1993). According to Ryvarden (1991), the Hymenochaetaceae

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constitutes a taxonomic family of polypores comprising nine genera namely ; *Coltricia, Phellinus, Inonotus, Phylloporia, Pyrrhoderma, Coltriciella, Hymenochaete* and *Aurificaria.* The first one, growing on roots are usually stipulated, while the following ones growing on trunks and branches are dimidiate to resupinated. The polypores belonging to this family are characterized by the presence or not in their hymenium or in their frame of very distinct cystids also called pointed bristles with thick wall, yellow or brown not encrusted (Wagner and Fischer, 2002).

These polypores develop on tree trunks and branches (Patouillard, 1900). They are characterized by resupinated, sessile Basidiome responsible for wood rot diseases, leading to desiccation of a wide range of tree species (Van der Kamp, 1991; Ryvarden and Gilbertson, 1993 ; Castello et al*.,* 1995). The Hymenochaetaceae family contains 610 species belonging to 48 genera (Piepenbring, 2015). In this family, the two most famous genera are the genera *Phellinus* and *Inonotus*. At the generic level, especially in *Phellinus* species which represents more than half of the total number of Hymenochaetales species, the traditional systematics based on anatomo-morphological analyses of this group is imprecise or even incomplete (Corner, 1991). This genus includes several species complexes and is generally considered to be a polyphyletic group (Ryvarden, 1991; Fisher, 1996). The number of species in the genus *Phellinus* in the broad sense was estimated at around 154 species (Larsen and Cobb-Poulle, 1990). But this genus now has more than 180 known species (Piepenbring, 2015). The discrimination between *Inonotus* and *Phellinus*, initially based on the spore color and then on the hardness and durability of the carpophore, now rests on the structure of the hypha system; dimitic in *Phellinus* with generative septate and non-curly hyphae but generally monomitic in *Inonotus* with generative curly hyphae.

Species of the genus *Phellinus* are found in all parts of the world where they break down hardwood or live parasitically on trees (Piepenbring, 2015). They grow on the roots or on the trunks of trees up to a height of 10 m and the weight of the basidiocarp can exceed 50 kg (Dai and Cui, 2011). However, in Burkina Faso, limited data exist on polypores. The first inventories were carried out in 2005, 2013 and 2017 respectively (Guissou, 2005; Bicaba, 2013 ; Nankoné, 2017). These inventories made it possible to collect several specimens belonging to the genus *Phellinus* sensu lato all over the country. But the identification of the different species remains incomplete. The present study was carried out in the Tin landscape dominated by *Parkia biglobosa* (Jacq.) Benth and *Mangifera indica* (Linn.) and in the Kou classified forest in the part dominated by *Anogeissus leiocarpus* (DC) Guill. and Perr., in the western part of Burkina Faso. This study aims to contribute to the knowledge of species of *Phellinus* genus in Burkina Faso. This is a pioneering study because it constitutes the first anatomo-morphological characterization of the genus *Phellinus* sensu-lato in natural forests in western Burkina Faso.

MATERIALS AND METHODS

Sample collection sites

The work of collecting polypores took place in the passage of Tin and in the classified forest of Kou.

The landscape of Tin (Figure 1) is located 12 km from Orodara on the Orodara-Djigouèra axis. This site does not appear to be the subject of scientific study, hence the lack of published scientific data. This landscape is located between latitudes 11° 04'-11°06'N and longitudes 04° 55-04°58'W. The average annual rainfall varies from 900 to 1100 mm. The vegetation is dominated by wooded and wooded savannas. Forest formations are for the most part linked to the presence of permanent to semi-permanent watercourses, the banks of which are severely degraded by human activity. The most frequent woody species were: *Parkia biglobosa*, *Khaya senegalensis, Acacia albida*, but also orchards of *Mangifera indica*, *Anacardium occidentale*, etc. The richness and diversity of the Tin landscape in woody species offer chances of finding polypores which are subservient there.

On the other hand, the classified forest of Kou located 15 km North-west of the city of Bobo-Dioulasso covers an area of 114 ha. The term Kou refers to the river that flows through the forest. The Kou forest presents an essentially flat relief. It belongs to the Sudanese phytogeographic domain (Guinko, 1984) ; it has an important character due to its floristic diversity. The forest inventory carried out by the PAFDK (Coulibaly, 2003) reveals nearly 200 species distributed in the different formations, are mainly savannas and gallery forests.

Sampling

The sampling was carried out following a modified version of the methodology used by Balezi (2013) which consists of collecting data along transects, inside plots and collecting along road axes. To do this, investigations were carried out along the axes leading to the sites. Prospecting and collecting missions were carried out from July to September between 2016 and 2019. At each site, samples were collected along the transects over 200 to 300 meters. Basidiomata belonging to the genus *Phellinus* were collected from forest species or wood with a knife (Small basidiome) or with a machete (large and leathery basidiome). The characteristics of each basidiome were noted. The health status of each host plant as well as the stage of decomposition of the substrate (wood) was also noted. Then, the geographical coordinates of the place of harvest were taken using the Global Positioning System (GPS) and a photograph of the basidiome on the substrate was taken. The samples are carefully stored in a harvest basket after wrapping in aluminum foil. A technical photograph was taken on the base camp on each sample bearing a label. The description of the macroscopic characters consisted of describing the morphological characters of the basidiome in the fresh state. It took into account the mode of growth, the habit, the shape, the consistency, the margin of the carpophore, the coating of the carpophore as well as its dimensions (diameter, thickness, projection), the characteristics of the hymeneal surface (tubes, lamellae, prickles and pores).

This description was made according to the description sheet for macrofungi from De Kesel et al. (2002) which has been simplified and adapted for the description of polypores. The characters described allow a first discrimination between the genera. The

specimens collected in the field were dried using an electric desiccator (Dorrex brand) for 24 h to serve as a database for further studies. The dried basidiomata were placed in hermetically sealed mini-grip bags. Each dried sample was stored in a cabinet according to the fungal genus to which it belongs. The microscopic studies were carried out on exsiccata. An optical microscope equipped with a drawing tube of the brand NIKKON H 550 S was used for this purpose. Different cutting techniques have been used depending on the type of carpophore. A longitudinal cut was made at the level of the hymenium or at the level of the weft for the search for bristles and hyphae and basidia. A scraping of the hymeneal surface was done for basidiospores.

The sections obtained were placed in a drop of 10% KOH in order to re-swell the structures and finally to check the coloring reaction. Congo Red Ammoniacal in 1% dilute ammonia solution was used to stain the cell walls. A few drops of Melzer's reagent were used to check for spore amyloidy. Anatomical structures (connective hyphae, skeletal hyphae, hymenial and / or weft setae, basidiospores, etc.), were drawn. All the anatomical structures drawn were measured using a micrometer (µm) ruler. Thus, the length (L) and width (W) of the basidiospores, setae, and basidia were measured. From measurements of the length and width of the basidiospores, the ratio (Q) of the spore (Q = L / l) was calculated using an Excel spreadsheet, thus highlighting the differences in measurements. This made it possible to determine the shape of the basidiospore, setae, cystidia, and basidia. The drawings produced were enlarged on A3 size paper and then traced on scalp paper on which the cystidia, basidiospores, basidia and the hyphal system were carefully grouped. Each scalp paper was scanned and stored in a photographic format in JPG format then the images obtained were processed to make them clearer.

RESULTS

Anatomo-morphological description

Phellinus cf. *igniarius*

Phellinus igniarius (L. Fr.) Quél. 1886, Ench. Fung., P.172
Synonyms : *Polyporus igniarius*, *Boletus igniarius*, *Fomes igniarius*, *Phellinus igniarius*

Morphological structures

Perennial basidiome is crusted, robust and ungulate with a more less smooth appearance. The old part is black, cracked in places and the rest of the basidiome is greyish, with woody pulpit and gray-orange color turning black in contact with KOH. The lining of the basidiome is corky and thin. The margin of the basidiome is circular and well delimited by a greyish band upwards and whitish towards the hymenium. Hymenium is porous with fine, tight pores. The hymeneal surface is rusty ocher to yellowish in color. The diameter of the basidiome is 17-20 cm, on a projection from the substrate of 10-16 cm and a thickness of 6-13 cm (Figure 2A and B)

Microscopic structure

The hypha system is dimictic, consisting of generative, compartmentalized, thin-walled hyphae and fairly thick-walled skeletal hyphae. The basidia: 25-40×10-18 µm, are cylindrical with keyed-out and a little pot-bellied. The setae: 90-65 ×15-20 µm are more or less long and have a somewhat thick wall. The bristles appear quite long and pointed at their apex under an electron microscope. The lining of the basidiome consists of thin skeletal hyphae with branched apices. Basidiospores : 8-5×6-4 µm, Q= (1.50-1.28-1.00 µm ; n= 60/2) are smooth, subglobose to globose (Figure 3a-f).

Ecology: The specimen was collected from the trunk of Anogeissus leiocarpus (African Job) at a height of about 2 meters. It is a parasitic species frequently found on this plant in the classified forest of Kou.

Material examined in Burkina Faso, Houet Province, Bobo Dioulasso. Samples no. NKS0152 (Holotype), coordinates: Latitude: 11°11'14.7"N., Longitude: 004°26'46.9"W., Altitude: 336 m. Harvested by NANKONE Samson, on 08/05/2018 in the classified forest of Kou.

This perennial species is characterized by a large, robust, ungulate and truncated, greyish basidioma with black and cracked old part. Hyaline Melzer spores are rare and small in size, turning orange to yellowish when in contact with KOH. The hymeneal setae are very long and tapered. It is a species collected for the first time in 2016 and then in 2018 on *Anogeissus leiocarpus* in the classified forest of Kou in western Burkina Faso.

Phellinus cf. *leavigatus*

Phellinus leavigatus (P. Karst.) Bourdot & Galzin 1928, Hym. France, p.624
Synonym : *Polyporus leavigatus* (Fr.), 1874

Morphological structure

Perennial basidioma is resupinated with a rigid flesh, brownish in color against the trunk of *Parkia biglobosa* at a height of nearly 2 m above the ground. The basidioma diameter is 8 cm, its basidioma thickness does not exceed 2 cm, and its spread is 16 cm^2. The hymenium is porous and concolorous at the basidioma. The pores line the surface of the basidioma, with approximately 10-12 pores / cm. The hymeneal surface is smooth and the pores are oval (Figure 4).

Microscopic structures

The hypha system is dimictic made up of generative hyphae and skeletal hyphae. Generative hyphae are septate with dichotomous branching, and skeletal hyphae

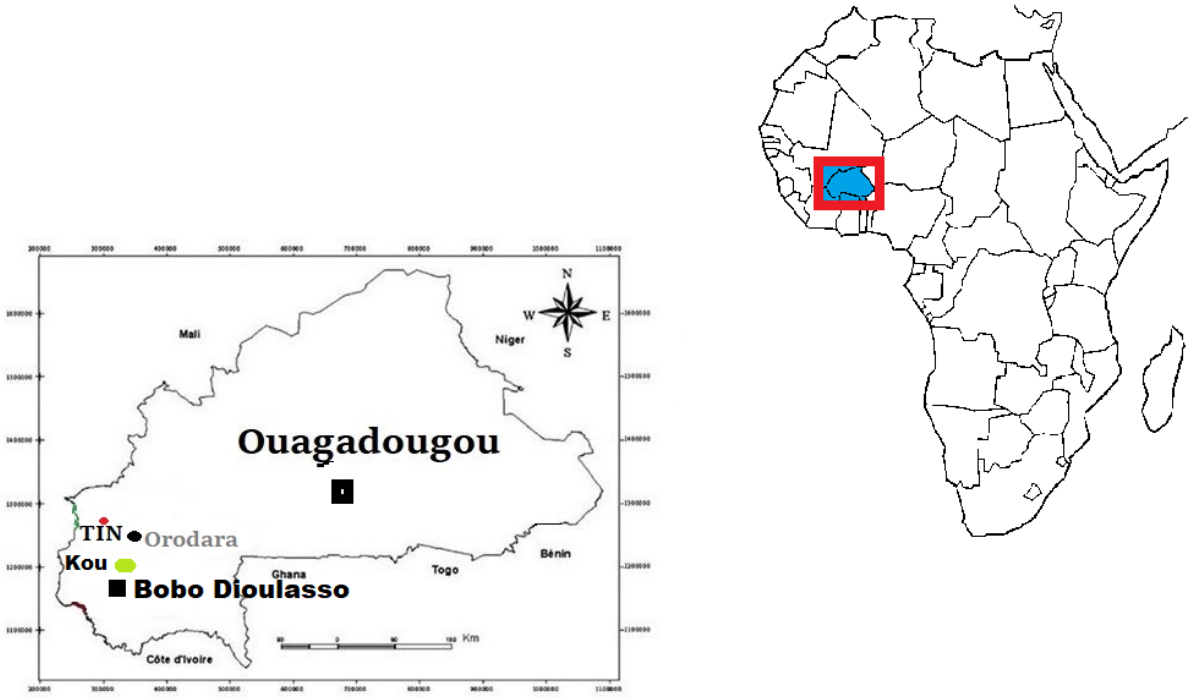
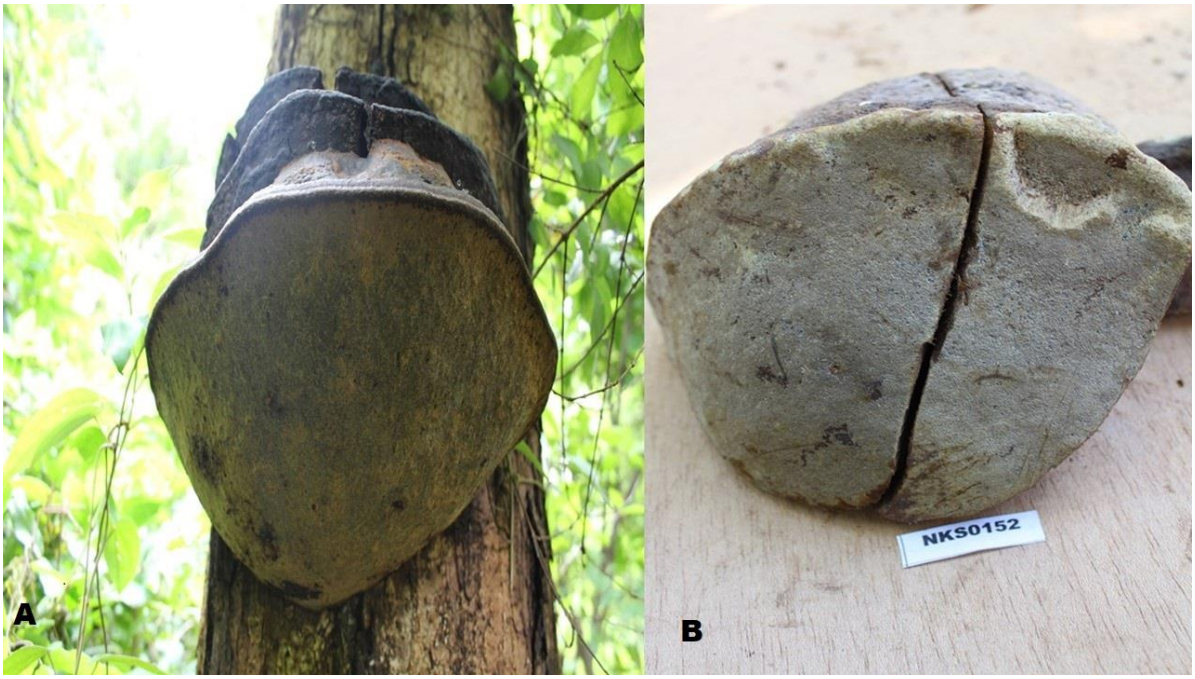


Figure 1. Location of landscape of Tin and the classified forest of Kou on the map of Burkina Faso. Source : NANKONE (2019).



Figures 2 A&B. Morphological structures of *Phellinus* cf. *igniarius*. **A.** *Phellinus* cf. *igniarius* in its habitat on the trunk of *Anogeissus leiocarpus* **B.** *Phellinus* cf. *igniarius* with a sample number.

are thick-walled and parallel in the weft. The basidia : 8-12×19-39µm are slightly keyed and cylindrical. Hymeneal setae : are not very thick, short with a more or less pointed and forked apex. Basidiospores : 8-5×6-4µm, Q=

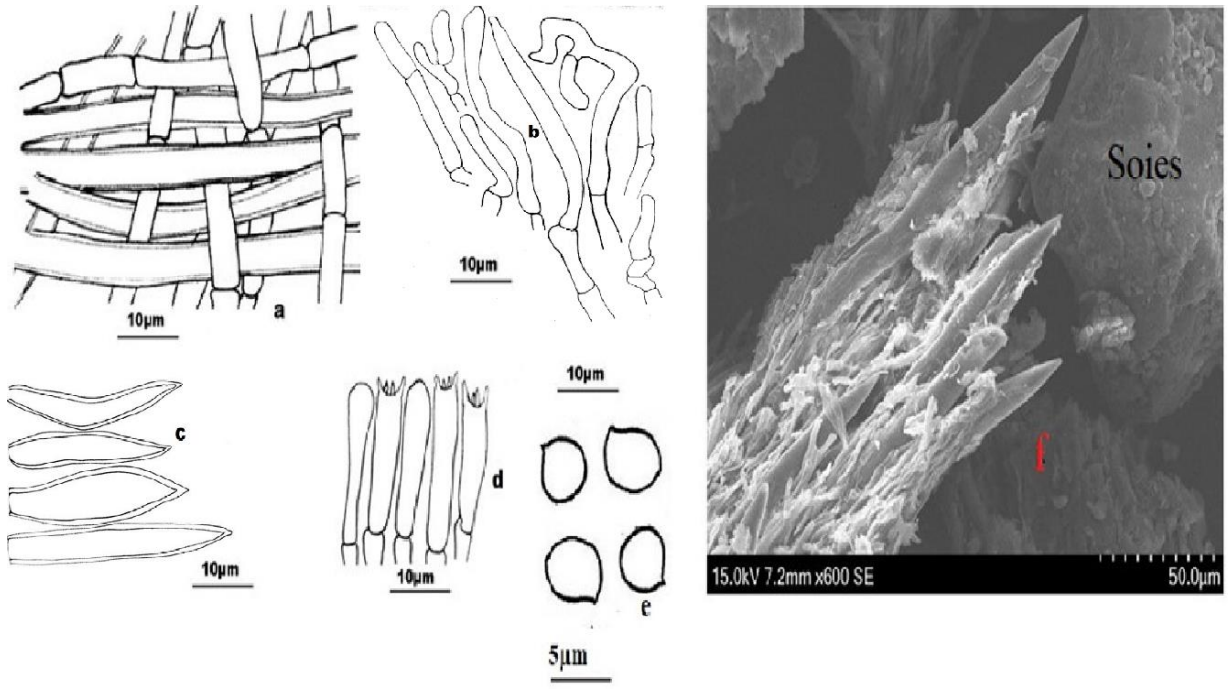
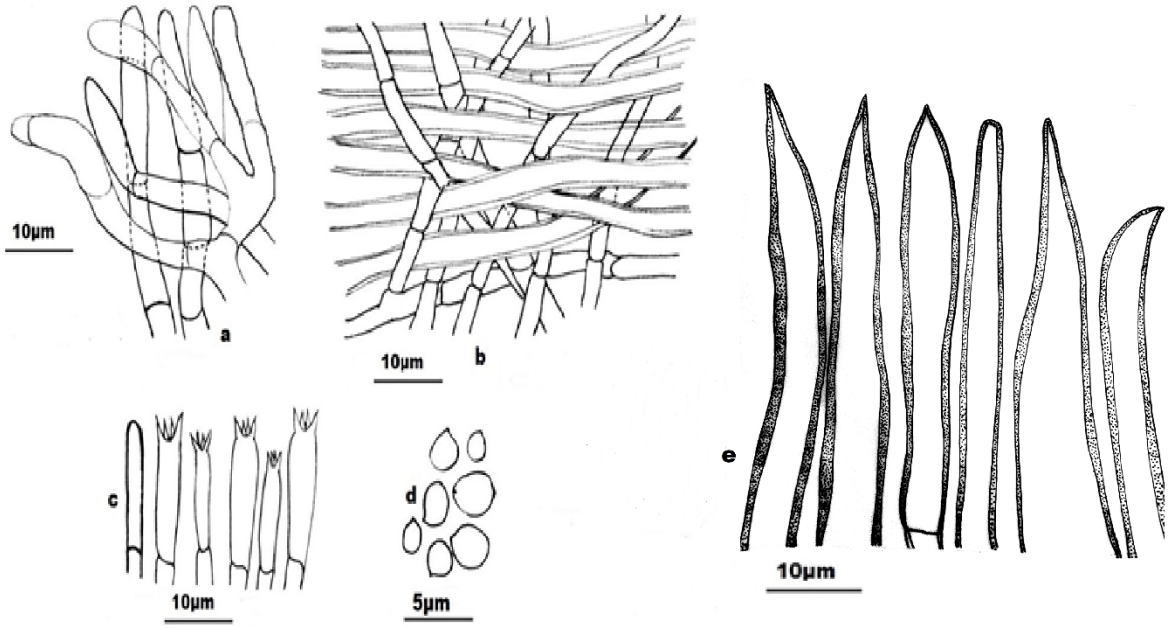


Figure 3a-f. Microscopic structures of *Phellinus* cf. *igniarius.* **a.** weft hypha system, **b.** Terminal cells of hyphae, **c-f.** hymeneal setae, **d.** basidia and little basidia and **e.** basidiospores.

Figure 4. Morphological structure of *Phellinus* cf. *leavigatus* in its habitat on *Parkia biglobosa* trunk.



Figures 5 a-e. Microscopic structures of *Phellinus* cf. *leavigatus.*
(a) generative hyphae, **b.** weft hyphae system, **c.** basidia, **d.** basidiospores and **e.** setae in the hymenium

(1.60-1.30-1.00, n= 30/1) are smooth and globular to subglobose (Figures 5 a-e).

Ecology: Perennial basidioma is collected from the trunk of *Parkia biglobosa* 2 m from the ground. It is responsible for the brown and white rot in wood.

Distribution: It is a cosmopolitan, pantropical species reported from Europe, Central Siberia.

Material examined: Burkina Faso. Province of Kénédougou (PTIN), no. NKS042 (holotype), coordinates : Latitude 10° 53'54.4"N, Longitude 004° 50'53.0"W and Altitude 457 m collected on 08/22/2016.

Phellinus cf. *leavigatus* was collected only once in 2016 in the Tin Landscape. It is a resupinated, olive-colored basidioma that is thin and less spreading. The spores are smooth globose to subglobose, becoming amyloid in Melzer. *Phellinus leavigatus* was collected only from *Parkia biglobosa*.

Phellinus cf. robustus

Phellinus robustus (Karsten) Bourdot and Galzin, 1925. Bull. Soc. Mycol. France, 41 : 188.
Synonym *Fomes robustus* (Karst.) 1889 (Figures 6 and 7).

Morphological structures

Large, perennial basidioma, oblong, greyish in color, the old part of which is black, cracked, cracked and presenting bulges. The margin of the basidioma is flattened and thick with a greenish color which turns brown over time and when touched or blackens in contact with KOH. The margin secretes a limpid exudate which darkens to the touch. The porous hymenium is rusty ocher in color consisting of fine and circular pores, the tubes are woody stratified and very sclerified with a length of up to 2-5 cm. The part in contact with the substrate is covered by a clearly visible whitish to yellowish (rhizomorph) down. Its diameter is 20-30cm, its projection is 15-30 cm, and its thickness is 10-15 cm (Figure 6A and B).

Microscopic structures

The hyphae system is a dimitic hyphae system made up of generative septate hyphae, and thick-walled skeletal hyphae. Generative hyphae are quite numerous with a rounded top. The basidia : 21-15×10-5 µm are stocky with rather long sterigmas. The setae : 170-25 × 24-9 µm are numerous and have a thick double wall. The tramal setae are long while the hymeneal setae are shorter with a curved apex. The basidiospores: 8-5 × 6-5 µm, Q = (1.60-1.31-1.00; n = 60/2) are smooth, subglobose to globose (Figure 7a-e).

Ecology: Perennial fruiting bodies collected living from *Parkia biglobosa*.

Material reviewed: Burkina Faso. Province of Kénédougou, Landscape of Tin (PTIN), sample number

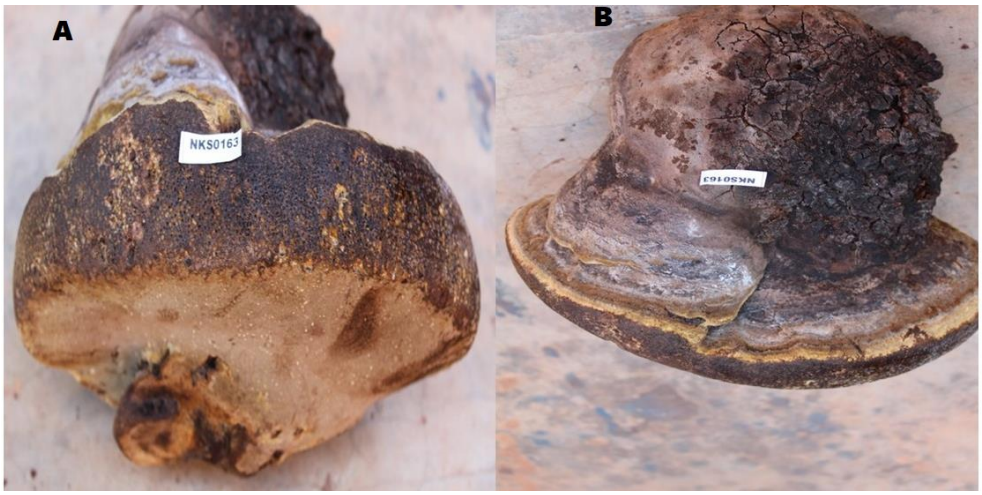
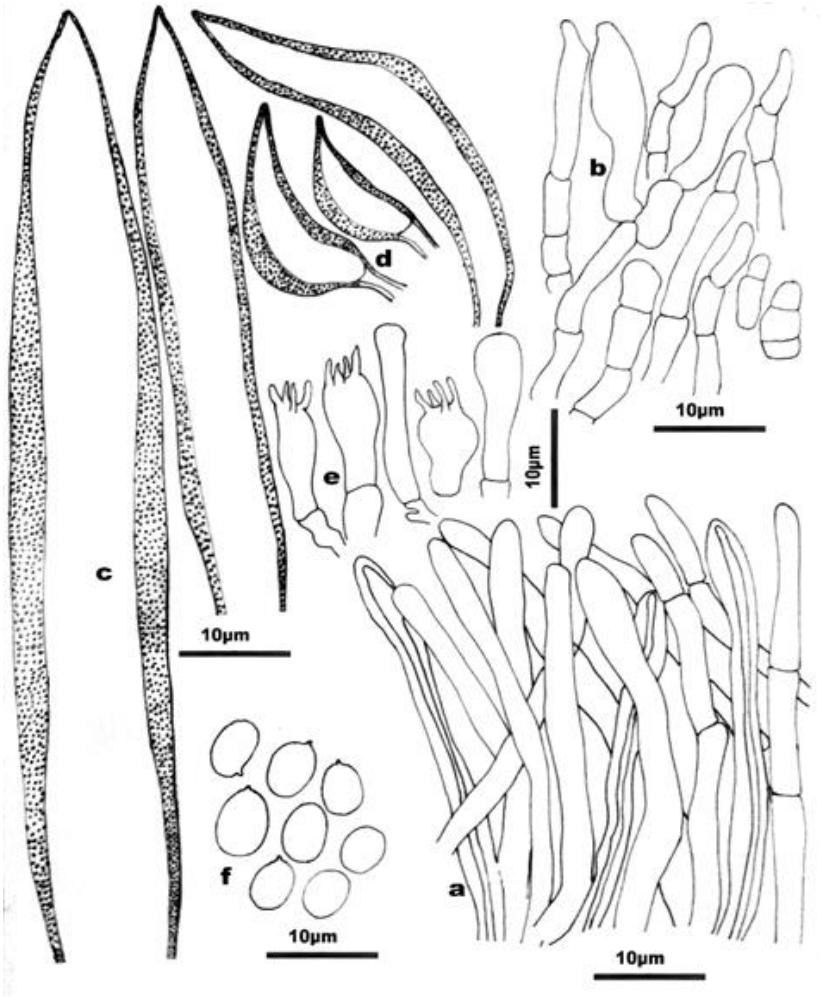


Figure 6 A&B. Morphological structures of *Phellinus* cf. *robustus.* **A.** general view of the hymeneal surface of the basidioma, **B**. overview of the surface of the basidioma.



Figures 7 a-f. Microscopic structures *Phellinus* cf. *robustus.* **a.** Generative and skeletal hyphae, **b.** Terminal cells of hyphae, **c.** long double-walled tramal setae with pointed tip, **d.** Short hymeneal setae with curved apex, **e.** basidia and basidioles, **f.** basidiospores (Scale = 10 µm).

NKS0163, collected on 5/08/2018, Coordinate: 11° 05'01.2"North; 004° 57'02.5" West, Altitude 527 m.

Phellinus cf. *robustus* is a perennial species, very robust, ungulate with a thick margin and the old part of which is black and cracked. The basidioma is greyish in color with a brownish flesh that turns black in contact with KOH. The basidiospores are smooth, small and hyaline. This specimen has only been found on *Parkia biglobosa* in Burkina Faso.

DISCUSSION

Systematic

In traditional taxonomy, the genus *Phellinus* sensu lato differs from other genera of the Hymenochaetaceae family only by a dimictic hyphal system and the consistency of the basidioma. However, the systematics based solely on anatomo-morphological analyses of this kind is imprecise (Corner, 1991; Fiasson and Niemelä, 1984; Wagner and Fisher, 2001, 2002). Nevertheless, this study is essential and for that, it constitutes the first characterization of fungal species and which is completed by molecular analyses. Based on the anatomo-morphological characters, the genus *Phellinus* Quél. is defined as a group of tawny, cinnamon, rusty polypores, etc., whose caps or basidioma are in console or sometimes resupinated sessile without distinct rind, perennial. These mushrooms have a porous hymenium, with layers of tubes, often without a distinct layer of flesh. These polypores have woody, rufous, reddish-brown flesh, with a dimictic hypha system, non-curly yellowish-brown hyphae, and usually bristles present in the hymenium. The spore of these polypores is white to rusty, the spores smooth, rounded to elliptical, non-amyloid, sometimes a little dextrinoid or cyanophilic and are responsible for the white rot of the wood. Polypores of the genus *Phellinus* can be found all year round but are only fertile in the right season (Patouillard, 1900). The morphological characters that allowed the discrimination of species of the genus *Phellinus* in this study are mainly the shape of the basidioma, the dimensions of the basidioma, the hyphal system, the length of the bristles, the shape and the size of the basidiospores. Three species have been described in the context of this study: *Phellinus* cf. *igniarius*, *Phellinus* cf. *leavigatus* and *Phellinus* cf. *robustus*. These three species are all characterized by a sessile basidioma of woody consistency and have a dimictic hypha system. We noted the presence of hymeneal setae and smooth spores in all three species.

Phellinus cf. *igniarius* is characterized by a robust ungulate and truncated, greyish basidioma with black and cracked old part, with globose to subglobose spores. The same species, *Phellinus igniarius* had previously been described as having an effusive-reflexed basidioma with basidiospores (4.5-6 × 5-6.5 µm) Niemelä (1975), Fisher and Binder (2004). It should be noted that *P. igniarius* groups together with a polyphyletic complex of species of the genus *Phellinus* whose main characteristics are: a dimictic hyphae system, setae always present in the weft and in the hymenium of non-dextrinoid and hyaline basidiospores (Lamrood and Goes-Neto, 2006). In the present study, the basidiospores (8-5×6-4 µm) appear relatively larger than those previously described by Niemelä (1975), Fisher and Binder (2004). Our results did not agree with these authors. *Phellinus* cf. *igniarius* has long, tapering setae. Such a remark was made by Tomsovsky et *al.* (2010) who showed that *P. igniarius* is characterized by long and tubular hymeneal setae.

Phellinus cf. *leavigatus* has a resupinated, perennial basidioma of thin olive color and less spreading, perennial with basidiospores : 8-5×6-4 µm, smooth and globular. This species had been described as a resupinated species having a dimictic hypha system with basidiospores, 3-4 × 4-5 µm small and smooth (Niemelä, 1972; Ryvarden and Gilberson, 1994). According to Patouillard (1900), the spores of *Phellinus leavigatus* have a thick wall, smooth, hyaline, sub-globular, 4-5×3.5-4 µm. This differs from our results which present larger basidiospores. *Phellinus* cf. *leavigatus* has thin hymeneal setae with a more or less pointed and forked apex. This presents a similarity to the description made by Robert (2011) that showed that *Phellinus leavigatus* is marked by an absence of setae in the weft but has rather short hymeneal setae with pointed tips often split. But this differs slightly from the description made by Patouillard (1900) according to which the hymeneal setae dark brown with thick walls, swollen base, top in "halberd", short, 10-20 × 4-8 µm.

Phellinus cf. *robustus* is also a perennial, very robust, ungulate to ungulate-reflexed species with a thick margin and the old part of which is black and cracked. The basidiospores of *Phellinus* cf. *robustus* are smooth, small and hyaline. However, *Phellinus robustus* is said to have dextrinoid and sub-globular basidiospores (Rajchenberg and Wright, 1987; Decock et al., 2005). Thus these basidiospores would have a strong affinity with the spores of *Phellinus elegans* (Robledo et al., (2006). According to Fiasson and Niemela (1984), *Phellinus robustus* would have globular, cyanophilic and dextrinoid spores. Our described specimen shows setae having a thinner double wall with a pointed apex. This aspect of the silks of this species had been mentioned by Karadelev et al. (2006) who showed that the *Phellinus robustus* hymeneal setae are distinct with slender and elongated apices and are apparently characteristic of all species of the *Phellinus robustus* complex.

Ecology and distribution

The genus *Phellinus* contains ubiquitous lignicolous, parasitic or saprophytic species of wood and trees and

causing white rots of wood (Van der Kamp, 1991; Ryvarden and Gilberson, 1993; Castello et al., 1995; Yombiyeni, 2014). They are pantropical species present in all regions of the world. But given the wide morphological variability of species of this genus, precise data on their distribution in tropical Africa remain insufficient.

In tropical Africa studies of the genus *Phellinus* sensu lato have been carried out and published by European mycologists. Among these researchers, we have Leif Ryvarden through publications Ryvarden and Johansen (1980), Masuka and Ryvarden (1993), Ryvarden (1998), Roberts and Ryvarden (2006) and Decock in publications Decock and Mossebo (2001, 2002), Decock et al. (2005), Decock and Bitew (2012). However, in Central Africa, this fungal genus has been mainly studied by Alphonse Balezi in Congo Balezi and Decock (2009), Balezi (2013) and Prudence Yombiyeni in Gabon (Yombiyeni et al., 2011 ; Yombiyeni, 2014). In West Africa, the only work devoted to polypores was carried out in Benin by Boris Olou, in 2020. This work comprehensively dealt with taxonomic studies based on the molecular phylogeny of polypores in tropical Africa (Olou, 2020). However, work in West Africa by local researchers has not specifically addressed the distribution of species in the genus *Phellinus*. The three species : *Phellinus* cf. *igniarius*, *Phellinus* cf. *leavigatus* and *Phellinus* cf. *robustus* which were the subject of this study were found in Burkina Faso on *Parkia biglobosa*, *Mangifera indica* and *Anogeissus leocarpus*. They are cosmopolitan species, present in both tropical and temperate climates (Ryvarden and Johansen, 1980). *Phellinus* cf. *igniarius* and *Phellinus* cf. *robustus* have been found in East Africa in two countries. Ethiopia and Tanzania Larsen and Cobb-Poulle (1990). *Phellinus igniarius, Phellinus leavigatus* and *Phellinus robustus* have been reported in Europe as parasitic species of deciduous trees such as *Quercus, Betula, Castanea, Robinia, Salix, Alnus*, and *Carpinus* etc (Karadelev et al., 2006). *Phellinus leavigatus* has been reported in Russia where it exhibited strong specialization on host genus *Betula* (Park et al., 2020).

Conclusion

Prospecting and collecting research of polypores in the classified forest of Kou and in the landscape of Tin permetted to collect three species of the genus *Phellinus*. They are: *Phellinus* cf. *igniarius*, *Phellinus* cf. *leavigatus* and *Phellinus* cf. *robustus*. These three species are all characterized by sessile basidiome with a dimictic hyphal system, with the presence of hymeneal setae and smooth spores. *Phellinus* cf. *igniarius*, a parasitic species of *Anogeissus leiocarpus*, is characterized by a robust ungulate and truncated, greyish basidioma with black and cracked old part. Its woody flesh is very rigid and of woody consistency. Its bristles are long, straight and tapered. However, *Phellinus* cf. *leavigatus* is a species collected from *Parkia biglobosa*. It has a resupinated, olive-colored basidioma that is thin and less spreading. Its basidiospores are smooth globular, becoming more or less orange on KOH. Finally, *Phellinus* cf. *robustus* has a perennial, oblong basidiome with a flattened and thick margin of greenish color turning brown with time and to the touch and bearing bulges. Its coating is greyish and turns black, cracked, with age. Its thick margin secretes a limpid exudate which turns black when touched. These three species were described for the first time in Burkina Faso. Therefore, their complete and exact identification requires molecular studies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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