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PhD Thesis

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Variation among *Acacia senegal* (L) Willd trees of different genetic origins in relation to climatic adaptation.

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Table of contents

Abstract

Predictions of climate change suggest a global increase in CO_2 and temperature in Africa, but rainfall patterns are still uncertain. *Acacia senegal* has received increased interest due to its derived gum arabic, drought tolerance, wide distribution, presence of multiple levels of ploidy and ability to fix nitrogen. To enhance knowledge on degree of genetic variation among populations in *A. senegal* with a view to its domestication and management in the Sahelian area, common garden trials in Senegal were explored in order to understand the morphological and phenological responses associated with the adaptation of the species to dry conditions. The thesis consists of three manuscripts.

The study presented in Manuscript 1 revealed three levels of ploidy namely diploid (2n), triploid (3n) and tetraploid (4n), but at highly different frequencies among populations. Leaf morphology and isotopic discrimination in *A. senegal* varied according to ploidy level and geographic origin. Triploid trees had a distinct leaf shape, and tetraploids displayed high $\delta^{13}C$ and low $\delta^{15}N$ values compared to diploids. Manuscript 2 showed that the variation among provenances in survival, height and diameter was significant and differences could be partly explained by the climate at their site of origin. In general, provenances from dry sites survived better at both sites. In addition, provenances from dry sites on average performed relatively poorer in height and diameter at the wetter site (Bambey) compared to the drier site (Dahra), while the opposite was the case for provenances from wetter sites. The study in Manuscript 3 indicated that *A. senegal* started the development of leaves prior to the beginning of the rainy season with flowering and fruiting initiation occurring during the rainy season. The phenology in *A. senegal* is under genetic control with significant differences observed among populations and ploidy levels. Variation in leaf phenology corresponded with local adaptation for diploid trees, but the same patterns was not found for tetraploid trees. Flowering phenology differed between diploid and tetraploid trees, but not to an extent that suggests it could provide an effective reproductive barrier between the two ploidy levels. Overall, the present thesis demonstrated that *A. senegal* across Africa consists of provenances that are genetically differentiated in their leaf morphology, phenology and growth, and these differences most likely reflect adaptation to local climatic conditions.

Summary

Acacia senegal is a multipurpose tree species widely distributed in Africa and Asia, and internationally recognized for its production of gum arabic. The species grows across a geographic range of mainly dry environments. The species has high drought resistance, and is considered as an important component in agroforestry systems due to its ability to fix nitrogen. It has multiple ploidy levels, and it has previously been reported that growth and response to drought can depend on the ploidy level.

To face the ongoing climate changes in Africa, species such as *A. senegal* are highly recommended in the Sahel, which is characterized by dry conditions where drought is the main factor affecting plant growth. It is therefore important to understand to what degree the species is locally adapted to the different environmental conditions within its natural distribution area. The thesis addresses this question by comparing trees that originate from areas with different climate in order to study how their performance depends on the climate of their site of origin. The aim is to enhance knowledge on degree of genetic variation among populations in *A. senegal* with a view to its domestication and management in the Sahelian area. The studies are based on two common garden trials that included 18 populations each of *Acacia senegal* at the establishement in 1994.

Based on flow cytometry, it was revealed that the frequency of diploids (2n) and polyploids (3n and 4n) varied substantially among the investigated (16) populations. Some populations included mixed levels of polyploidy, but populations with only tetraploid trees and populations with only diploid trees were also observed.

Leaf morphology, total leaf nitrogen and isotopic discrimination varied significantly according to ploidy level and geographic origins. Diploids (2n), triploid (3n) and tetraploids (4n) were significantly differentiated in their leaf morphology, with particularly triploids having distinct shape. Triploid trees were removed from the analysis because it could not be excluded that they were of hybrid origin. Tetraploid individuals displayed high carbon isotope ratio ($\delta^{13}C$) and low nitrogen isotope ratio ($\delta^{15}N$) compared to diploid individuals. Among diploid trees, leaf length and number of leaflets varied with annual rainfall and latitude, respectively, with populations from dry

sites having small leaves and a larger number of leaflets per leaf. The findings suggest that the differences likely reflect adaptation to different climatic conditions, but the patterns tend to differ between diploid and tetraploids.

Genetic variation in survival, and growth (height and diameter) among 15 African provenances was investigated after 14 years at two sites (Dahra and Bambey; Senegal), and 23 years at one of the sites (Dahra). Survival, height and diameter differed significantly among provenances and could be partly explained by the climate at their site of origin. The comparison of the two trials revealed that provenances from dry sites in general survived better at both sites. However, in terms of diameter and height, provenances from dry sites on average performed relatively poorer at the wetter site (Bambey) compared to the drier site (Dahra), while the opposite was the case for provenances from wetter sites. The observation of genotype-by-environment interaction combined with differences among provenances that could partly be explained by climate conditions, support that divergent selection creates and maintains local adaptation of *A. senegal* provenances. Breeding programs and forest restoration projects should therefore carefully select germplasm for optimal performance depending on the planting site of the socioeconomically important species.

The leafing, flowering and fruiting phenology of *A. senegal* trees at the common garden trial Dahra were monitored during 17 months and observations were compared to the rainfall during the period. Investigation of variation in timing of the phenological phases within and among provenances showed that *A. senegal* can initiate buds before the onset of the rain at the common garden, while the development of leaves starts at the beginning of the rainy season with flowering and fruiting initiation occurring during the rainy season. Significant differences were observed among populations, and primarily among ploidy levels in these traits, revealing that phenology in *A. senegal* is under genetic control. In general, early leaf flushing trees had a longer growing period and performed better in terms of growth at the tested site. The results for diploid trees indicated that the leaf phenology reflected local adaptation, but the same patterns was not observed for tetraploid trees. Flowering phenology differed between diploid and tetraploid trees, but not to an extent that suggests it could provide an effective reproductive barrier between the two ploidy levels.

The overall finding of the thesis is that *A. senegal* across Africa consists of provenances that are genetically differentiated in their leaf morphology, phenology and growth. Moreover, these differences partly reflects adaptation to local climatic conditions. The results has important implication for sustainable use and management of genetic resources of the species given the uncertainty of future climate.

Sammendrag

Acacia senegal er en træart i ærteblomstfamilien (Fabaceae), som vokser naturligt i tørre områder i Afrika og Asien. Den nøjsomme art er en vigtig del af landbrugssystemerne i mange områder (agroforestry), men arten er også kendt, fordi den producerer gummi arabicum, et produkt som eksporteres til fødevareproduktion i en række lande, inkl. Danmark. Arten har således stor betydning både lokalt, nationalt, regionalt og internationalt. Alligevel ved man relativt lidt om arten. For eksempel er det kun få år siden, at man opdagede, at arten har flere ploidi-niveauer, hvilket vil sige, at der er forskelle i, hvor mange sæt kromosomer som træerne har. Nogle har to, andre har tre, fire eller endnu flere sæt. Disse forskelle har betydning for, hvordan de enkelte træer vokser og klarer tørkestress. Og selvom arten vokser flere steder i meget tørre områder, er der også begrænset viden om hvorvidt – og i givet fald hvordan - *Acacia senegal* træer fra disse områder er tilpasset særligt tørre vækstbetingelser.

Viden om artens tilpasning til lokale vækstbetingelser er vigtig, når man skal vælge og udvikle frøkilder til plantningsprogrammer. Det er afgørende, hvis man vil øge planting med *Acacia senegal*, f.eks. for at øge kulstofbinding eller for at reducere ørkenspredning. Og det er særlig vigtigt på grund af de delvist uforudsigelige klimaændringer, som i værste fald kan ændre vækstvilkårene markant, ikke mindst i de områder, hvor der i forvejen er meget beskeden nedbør.

I denne afhandling undersøges spørgsmål om klimatilpasning ved at sammenligne *Acacia senegal* træer, som stammer fra 16 forskellige områder i primært Afrika. I 1994 blev der plantet to forsøg i Senegal med frø indsamlet de 16 steder, og det er træerne i disse forsøg, som danner fundamentet for analyserne i denne afhandling. De fleste analyser er baseret på målinger på de 23 år gamle

træer i et af forsøgene, mens et af studierne primært benytter data fra målinger foretaget i begge forsøg, da træerne var 14 år gamle.

I artiklen '*Leaf morphology and stable isotope ratios of carbon and nitrogen in* Acacia senegal *(L.) Wild trees vary with climate at the geographic origin and ploidy level'* undersøges træernes ploidy-grader. Analyserne viser, at forekomst af diploide (2n), triploide (3n) og tetraploide (4n) træer varierer betydeligt mellem de 16 oprindelser. Nogle oprindelser har træer med forskellige ploidi-grad, men der er også oprindelser hvor alle træerne er enten tetraploide eller diploide. Studierne af bladene viser, at forskellene i ploidi-graden er afspejlet i bladenes udseende. Det er især de triploide (3n) træer, hvor bladene afviger i udseende fra 2n og 4n træer. De morfologiske forskelle er interessante, fordi blades opbygning kan påvirke træernes evne til at vokse under tørre forhold. Der er også forskel i bladenes sammensætning af kulstof ($\delta^{13}C$) og kvælstof ($\delta^{15}N$) isotoper, hvad forventes at afspejle forskelle i deres udnyttelse af vand og kvælstof. Det tyder også på, at variationen mellem oprindelser i træernes bladform ikke er tilfældig, men derimod afspejler tilpasning til forskellige klimatiske forhold. Træer fra tørre områder har generelt mindre blade bestående af mange småblade. Det gælder dog kun de diploide træer. Der ses ikke samme tydelige sammenhæng hos de polyploide træer.

I artiklen '*Survival and growth of* Acacia senegal *(L.) Wild. provenances in Senegal: adaptation to local climatic conditions and genotype-by-environment interactions'* undersøges træernes overlevelse og vækst på de to lokaliteter efter 14 år. Analysen suppleres med vækstdata 9 år senere fra en af lokaliteterne. Studiet viser, at der er stor forskel mellem de forskellige geografiske oprindelser i overlevelse og vækst. Også her tegner der sig et mønster: Træer fra tørre områder overlever generelt bedst. I forhold til vækst er billedet mere nuanceret, fordi resultatet påvirkes af, om træerne vokser på den tørre og sandede lokalitet (Dahra), eller på den lidt mere våde lokalitet (Bambey). Træer fra de tørreste områder vokser relativt bedst i Dahra, mens det omvendte er tilfældet i Bambey. Når man sammenligner vækst og overlevelse efter henholdsvis 14 og 23 år, er der ikke de store ændringer. Det ser således ud til at man i det store og hele kan stole på resultater efter 14 år. Der er dog antydning af, at en af de eksotiske oprindelser først begynder at klare sig dårligere ved den seneste måling. Resultaterne peger samlet på, at træerne er tilpasset klimaet på deres oprindelsessted.

I artikelen '*Variation in phenology of* A. senegal *(L.) Wild. in relation to origin and ploidy level - implications for climatic adaptation'* præsenteres resultater fra systematiske observationer af *A. senegal* træernes fænologi over 17 sammenhængende måneder. Arten er løvfældende, fordi den vokser i områder med markant tørtid. Når tørtiden slutter, springer træerne ud, blomstrer, producerer frugter og smider så bladene, når tørtiden igen begynder. Studiet viser, at det dog ikke er så simpelt, at træerne blot springer ud, når regnen kommer, idet udviklingen af knopper og det begyndende løvspring sker, inden den første regn er faldet. Desuden begynder træer fra nogle områder udviklingen før andre. Ved at sammenligne med træernes vækst viser studiet også, at det generelt er de træer, som har den længste periode med blade, som har vokset mest. Samlet set peger studiet på, at timing af udspringspunkt også er resultat af tilpasning til lokale forhold, således at træerne kan opnå den længst mulige vækstperiode uden at springe ud for tidligt og derfor lide tørkeskade. Det er velkendt, at træer på nordlige og sydlige breddegrader tilpasser deres årsrytme efter årstider baseret på registrering af temperatursum og daglængde, men det er stadig uklart hvordan tilpasningen sker i tropiske arter tæt på ækvator. Studiet af *A. senegal* giver ikke svar på det spørgsmål, men peger på at det er et emne der bør undersøges nærmere i de kommende år. Artiklen påviser også, at der er små forskelle mellem diploide og polyploide træer i deres fænologi. Blomstringsfænologi synes dog ikke at have stor betydning for opretholdelse af de forskellige ploidigrader, da der er overlap mellem diploide og polylpoide træers blomstring.

Samlet set viser studierne i denne afhandling, at der er genetisk differentiering mellem forskellige oprindelser af *A. senegal* i en række egenskaber, som må formodes at være vigtige for artens evne til at vokse under tørke-stressede forhold. Disse forskelle skyldes sandsynligvis, at de forskellige oprindelser gennem naturlig selektion er blevet tilpasset deres lokale vækstvilkår. Afhandlingen peger på behovet for langt mere forskning i træers tilpasning i Sahel. Denne viden vil være central i forhold til at understøtte plantningsprogrammer og klimatilpasningsaktiviteter.

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Objectives of the study and overview of the thesis

This thesis is about *Acacia senegal*, a species of major ecological and socio-economical importance, and widely distributed in the dry tropical regions of Africa and Asia. *Acacia Senegal* is expected to become influenced by climate change to which the species could respond through morphological and physiological changes. A combination of warming and changing in patterns of rainfall can particularly lead to a modification of the composition of vegetation in dry regions of Africa. However, *A. senegal* is of special concern because it already grows on very marginal lands in terms of water and nutrients availability, and it is urgent to understand the adaptive potential of the species in order to keep up with the pace of current climate changes.

Another reason of concern is the socioeconomic importance of *A. senegal* mostly generated by its derived gum arabic, which represents an important source of income for rural populations in Africa and particularly in the Sudano-Sahelian zones (Fagg and Allison 2004; Mujawamariya and D'Haese 2012). Gum arabic is a dried natural exudation of *A. senegal* trees, which is widely used in food, pharmaceutical and cosmetic industries globally. Apart from gum arabic, *A. senegal* is also known for various other products such as livestock fodder and fuel wood, and its ability to fix nitrogen. Its ability to tolerate drought impacts makes it an essential component of dryland agroforestry systems (Fagg and Allison 2004; Raddad et al. 2006).

This led to a large-scale recommendation of *A. senegal* in reforestation programs in Sahel to prevent future climatic change. Hence, efforts have been made to study the adaptive potential of the species to different sites using common garden trials previously (Ræbild et al. 2003a,b,c; Raddad and Luukkanen 2006; Raddad 2007; Larwanou et al. 2010; Gray et al. 2013; Diallo et al. 2016; Sarr et al. 2021). These studies showed genetic variation among natural populations of *A. senegal* that suggests a local adaptation to environmental conditions. Further, new insights on the genetics of the species revealed the presence of different levels of ploidy (number of sets of chromosomes) (Assoumane et al. 2012; Odee et al. 2015; Diallo et al. 2015), which is known to affect the species' ability to grown under dry conditions (Diallo et al. 2016; Sarr et al. 2021). However these later studies only concerned local populations of *A. senegal* from Senegal, and the ability of polyploids to grow under dry conditions has not been studied based on populations across the entire distribution of the species, and the relationship to leaf morphology and phenology remains unknow.

The overall objective of the present thesis is to contribute to the enhancement of knowledge on genetic variation in *A. senegal* for important quantitative traits with a view to its domestication and management in Sahelian region.

Specifically the study is divided into three manuscripts with specific objectives:

1. To test if and how ploidy level and climate at site of origin influence leaf morphology, total leaf nitrogen and composition of stable carbon and nitrogen isotopes of *A. senegal*.
2. To investigate the differences in survival and growth of trees from African provenances of *A. senegal* tested in two environments in Senegal, and to analyse potential Genotype-by-environment (G×E) interaction.
3. To explore genetic variation in phenological events of *A. senegal* trees in relation to their geographic origin and ploidy level.

Correspondingly, the main hypotheses in these studies are:

- Variation in leaf morphology in *A. senegal* reflects adaptation to the place of origin and/or is related to the level of ploidy.
- Differences in growth performance of provenances reflect adaptation to the climate at their site of origin, and these differences in adaptation lead to G×E interactions when the provenances were grown at sites with different levels of water stress in *A. senegal*.
- Variation in phenology in *A. senegal* can be related to the climate at the origin of the population. Also, phenology differs according to ploidy level where differences in flower phenology can contribute to a reproductive barrier between diploid and tetraploid individuals.

The thesis consist of four parts: (i) a general introduction that stated an overview of *A. senegal*, its natural ecosystem and uses, its adaptation to dryland ecosystem, and its genetic implication in breeding programs, (ii) the summary of material and method and results, (iii) a general discussion and limits of the study (iv) conclusion and perspectives for future research.

Introduction

Acacia senegal, its natural ecosystem and its use

Acacia senegal (L.) Willd. is a multipurpose leguminous tree species belonging to the genus *Acacia*, family Fabaceae and sub-family Mimosoideae. It was recently transferred to the new genus *Senegalia* which conferred it the new name *Senegalia senegal* (L.) Britton (Kyalangalilwa et al. 2013). Here we will maintain the rule of first priority and the name *A. senegal* will be used through the study. The species includes four varieties that differentiated morphologically, namely vars. *senegal*, *kerensis*, *rostrata* and *leiorhachis* (Fagg and Allison 2004). *Acacia senegal* has a wide natural distribution across Africa from the Sahelian belt to southern Africa (Fagg and Allison 2004), and is also found in India and Pakistan. The four varieties also vary in their distribution, *rostrata* and *kerensis* are respectively found in Southern and Eastern Africa (Fig. 1), and *leiorhachis* grows in both in the arid north east of Kenya and southern Somalia and in Tanzania (Fagg and Allison 2004). The variety *senegal* is the most widespread and the only found in Senegal, and also the main gum arabic producing tree species in Sudano-Sahelian regions (Fagg and Allison 2004, Raddad and Luukkanen 2006).

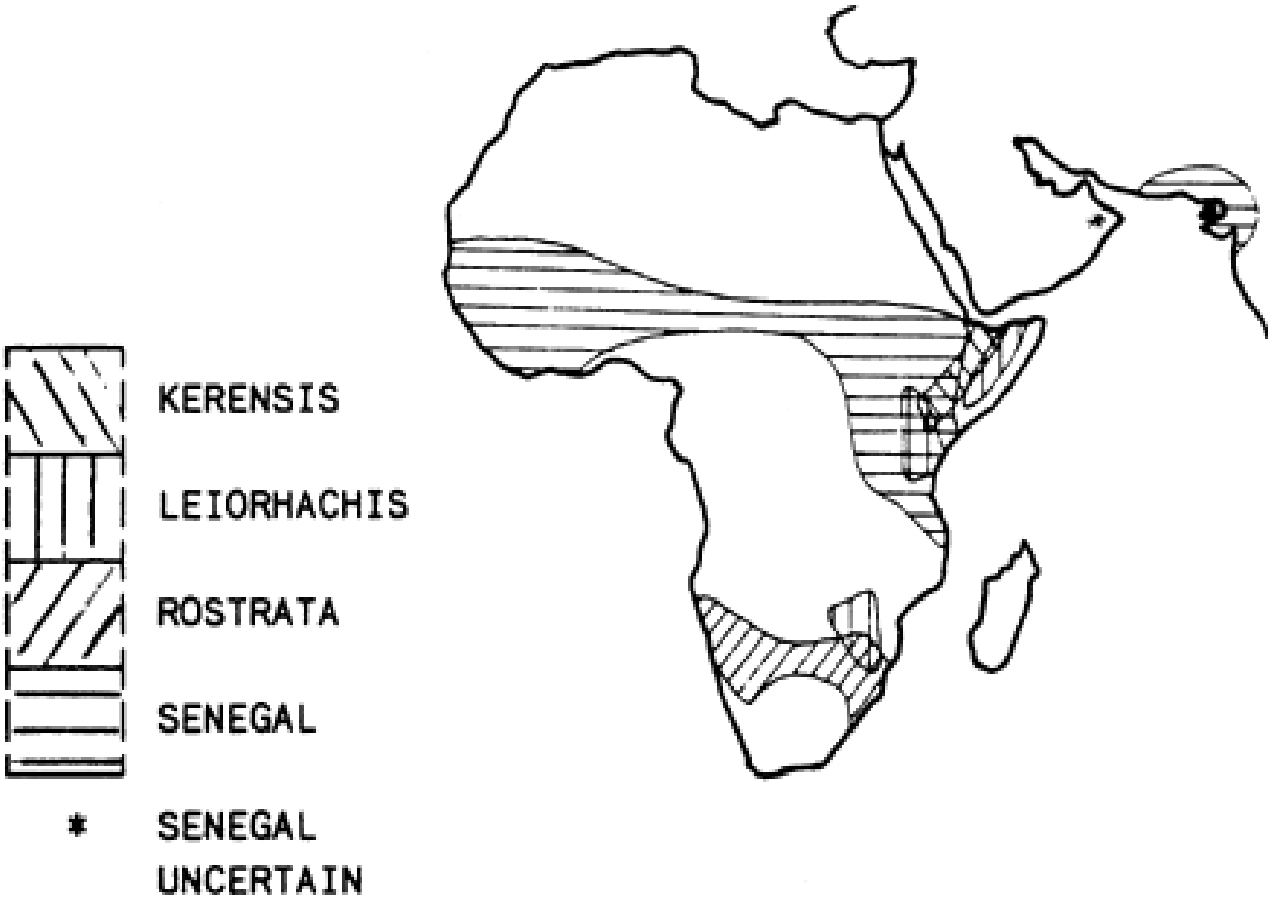

Fig. 1 Natural distribution range of *A. senegal* and its different varieties (Source: Brenan 1983)

Acacia senegal is a drought resistant species that is adapted to arid zones and usually occurs in areas with low soil fertility and rainfall varying between 200-800 mm (Fagg and Allison 2004). The species consists of a deep rooting system with a large allocation of biomass to roots (Raddad 2007; Merine et al. 2014), and constitutes an important component in dryland agroforestry systems providing fodder for animals and fuel wood, and improving soil fertility by its capacity to fix nitrogen (Fagg and Allison 2004, Raddad and Luukkanen 2006). Furthermore, *A. senegal* is internationally recognized as the main source of gum arabic production. The gum is a natural exudation obtained from stem and branches of *A. senegal,* which is used as a food additive in beverages and as an emulsifier and coating agent in industries (Mujawamariya and D’Haese 2012).

The pollination of *A. senegal* is mainly by bees (Tandon et al. 2001; Sprent et al. 2010), and the species is self-incompatible and a preferential outbreeder (Tandon et al. 2001). The phenological events of *A. senegal* correspond to the seasonality of the year (Tandon et al. 2001). The inflorescence of *A. senegal* consists of small hermaphroditic flowers with creamy white anthers (Tybirk 1993; Diallo et al. 1997; Fagg and Allison 2004). The species starts to flower at young age, i.e. around three years under normal conditions, and flowering usually occurs before or after the rains depending on the taxa (Fagg and Allison 2004). The rate of pollination in *A. senegal* is high with 52% of the stigmas pollinated but flowers are often aborted (Diallo et al. 1997), which is due to the competition for resources in the inflorescence and seed predation limiting the survival of the seeds (Tybirk 1993). The number of produced seeds per pod is on average 3 to 4.5, and seed weights vary considerably across the natural range (Fagg and Allison 2004).

Acacia senegal regenerates naturally through its natural distribution range and the success of the regeneration requires favorable environmental conditions (Fagg and Allison 2004). Planting activities in Africa mainly concern gum production with seeds from local provenances within the countries concerned (Fagg and Allison 2004). In addition, a series of provenance trials was established in order to find the best provenances in terms of adaptability and gum production (Ræbild et al. 2003a,b,c).

The African dryland ecosystems as habitat for *Acacia senegal*

Acacia senegal principally occurs in the arid and semi-arid regions of Africa in south of the Sahara, throughout the Sahelian zone from Senegal to Somalia (Assoumane et al. 2012). These ecosystems are characterized by large variability in rainfall, soil types from poor sandy soils to sandy clays (Fagg and Allison 2004). In the Sahel drylands of sub-Saharan Africa, rainfall and aridity variability increase with latitude (Mortimore 2010), i.e. annual rainfall varying from 150-200 mm in the north to 800 mm in the south with a single short growing season of 3 to 4 months, and dry winds contributing to a long and severe dry season (Fagg and Allison 2004). Thus, soil water availability is the major factor limiting survival, growth productivity of plants (Otieno et al. 2005; Khan et al. 2010), and species composition and distribution (Otieno et al. 2005) in dryland regions. Plants develop different strategies under water deficit conditions, ranging from drought-avoidance to drought resistance that vary with the genotype (Chaves et al. 2002; Aroca 2012). As a response to drought stress, drought tolerant species reduce water loss through stomata regulation that limit the rate of photosynthesis (Levitt 1980). This adaptive strategy is likely to influence plant survival and productivity (Padilla and Pugnaire, 2007; Olmo et al. 2014). Improving the water use efficiency (WUE) of drought adapted species such as *A. senegal* is of high interest.

The physiological adaptation of *Acacia senegal* trees to growth under dry conditions

Deciduous tree species such as *A. senegal* have a greater tolerance to drought stress thanks to their ability to use water and nutrients efficiently under dry conditions, and a relatively large allocation of biomass to roots (Raddad and Luukkanen 2006; Raddad 2007; Gray et al. 2013; Merine et al. 2014). In addition, *A. senegal* develops a deeper rooting system (Fagg and Allison 2004) that can increase the efficiency of soil exploration and water uptake (Poorter et al. 2012). *Acacia senegal* is an opportunistic water and nutrients use tree that growths faster when water conditions are suitable, and sheds its leaves under unfavorable conditions (Gebrekirstos et al. 2011; Merine et al. 2014). This suggests that the species grows mainly during the rainy season exhibiting morphological plasticity, higher vigor and investment in biomass production (Merine et al. 2014). In comparison to other drought tolerant species such as *Acacia tortilis* and *Balanites aegyptiaca*, *A. senegal* displayed a lower WUE under drought stress (Gebrekirstos et al. 2011) with lower growth.

Processes that influence fitness and drivers of local adaptation

The potential of *A. senegal* to adapt under increasing harsh conditions is not yet fully understood. Plants with higher WUE may extend the growing season when a long dry period occurs which can confer an ecological advantage in dryland ecosystems (Aroca 2012). Water use efficiency has been found to vary among and within populations of *A. senegal* as a consequence of local adaptation in growth and gum production. Raddad and Luukkanen (2006) reported that populations from sandy soil displayed more conservative water use and high drought tolerance compared to populations from clay soil, as a result of less productivity when grown at clayey site in Sudan. In Senegal, Gray et al. (2013) confirmed that *A. senegal* employs different WUE strategies in relation to the environmental conditions of the plantation site, but also with the age of the trees. Furthermore, Sarr et al. (2021) highlighted the effect of ploidy level on WUE at 15% of significance assuming the interference of other factors. While in a recent study (see Manuscript 1), WUE in *A. senegal* is found to vary according to ploidy level and geographic origin suggesting an adaptation to different environments, but patterns tend to differ between cytotypes (i.e. plants with different ploidy levels). This genetic trait is known to play key role on the species' ability to thrive under drought conditions, with polyploid individuals of *A. senegal* having superiority in growth compared to the diploid ones (Diallo et al. 2016). These results suggested that the existence of different ploidy levels in *A. senegal* can influence fitness in the species and increase its adaptive potential to future climatic fluctuations. In addition, in certain regions of African drylands it is possible to increase drought tolerance with drought superior genotypes.

Expected climate change and its likely effects for trees in the African *Acacia senegal*

Sahelian ecosystems have already been severely affected by recurrent drought events (Khan et al. 2010; Brandt et al. 2014; Taylor et al. 2017). In addition, climate model projections suggest a global increase in CO_2 and temperature and substantial reduced precipitation in some regions (IPCC 2020), and future water deficits in plants and crops are therefore a serious concern. Increasing temperatures will increase the rate of water use of plants due to the effect of temperature on vapor pressure deficit, while increasing CO2 will decrease stomatal conductance, leading to increased water-use efficiency (WUE), but this will vary with species and will depend on soil water status (Hatfield and Prueger 2011). However, it is important to note that there is uncertainty in expected rainfall patterns including both seasonal declines and increases, which require ongoing review of climate trends and their causes (Chapman et al. 2012). To face with ongoing climate change, understanding the morphological and phenological responses associated to the adaptation of *A. senegal* in dry conditions, can be useful for its domestication in dryland areas.

Studies of genetic variation within and among populations of *Acacia senegal*

Genetic variation among different origins of *A. senegal* has been reported in several studies by investigating common garden trials across Africa. In Burkina Faso, Ræbild et al. (2003a,b,c) found large variation in growth and survival among *A. senegal*, with a dominance of Sahelian provenances compared to the Sudanian provenances, and weak performance of a provenance from India. In Sudan, Raddad and Luukkanen (2006) explored a trial located in a clayey site, and showed genetic variation in growth, carbon isotope composition and gum production between *A. senegal* provenances from clayey and sandy soil areas of the gum belt in Sudan. Still in Sudan, Raddad (2007) reinforced the later differences by demonstrating genetic variation in seed morphology and seedlings traits between both types of provenances from clay and sandy soils. When assessing a mature trial consisting of 11 provenances from Niger, Mali and Sudan in Niger, Larwanou et al. (2010) found that provenances from Mali and Niger performed better in survival, height growth and basal area, which correlated with the rainfall and geographical coordinates of the origins. Investigation between two provenance trials located in different environments in Senegal reported that survival and growth of *A. senegal* trees depended on genetic origin, and that the variation among the provenances could be partly explained by the climatic conditions at their sites of origin (Diatta et al. 2021a). In this later study, provenances that originated from dry sites in general survived better than provenances from wetter sites at both sites. In addition, provenances from wetter sites generally ranked better as regards diameter growth at the wet site while provenances from dryer sites in general ranked better at the dry site, implying the presence of genetic-by-environment interaction (G × E) in diameter.

The recent discovery of polyploidy in *Acacia senegal*

New insight on genetic variation emphasizing the occurrence of polyploidy (more than two sets of chromosomes of an organism) in *A. senegal*, has been recently discovered by molecular marker analysis. Assoumane et al. (2012) reported first the presence of both diploid and tetraploid *A. senegal* individuals, and later Odee et al. (2015) and Diallo et al. (2015 and 2016) showed the presence of triploid, tetraploid, pentaploid and hexaploid individuals across its natural range in Africa, India and Pakistan. In East African populations from Ethiopia (Sodera) only tetraploid individuals were discovered (Odee et al. 2015; Diatta et al. 2021) perhaps suggesting the absence of mixed ploidy populations in the southern range of the species. Diallo et al. (2016) have found that polyploidisation plays an important role in the ability of *A. senegal* to grow under harsh conditions with polyploid individuals growing faster than diploids ones when subjected to drought stress, and that the presence of multiple ploidy levels therefore increases its adaptive potential.

Evidence for presence of local adaptation in *Acacia senegal?*

Variation among populations is not always a result of local adaptation. Local adaptation of a species occurs when spatial variation in environmental conditions enforces divergent selection among populations across its natural distribution area (Wadgymar et al. 2017). It can be a result of differentiation in physiological and morphological traits as response to divergent natural selection (Eriksson et al. 2020), or a consequence of polyploidisation (Diallo et al. 2016). The observed genetic variation in survival and growth of *A. senegal* populations suggests an adaptation to local climatic conditions. Indeed, the Sahelian provenances had advantage in growing in a dry site in Burkina Faso compared to the Sudanian provenance (Ræbild et al. 2003a,b,c), and clayey provenances outperformed sandy provenances in a clay site in Sudan (Raddad and Luukkanen 2006; Raddad 2007. However, the performance of the different genotypes have been investigated in a particular environment and with a restricted number of provenances (Diatta et al. 2021b). Exploring the same provenances at multiple sites will contribute to the understanding of the genetic and environmental mechanisms that contribute to local adaptation in *A. senegal* and may have implications for breeding and planting programs.

Domestication and breeding

The ecological and socio-economical importance combined with presence of genetic variation in important traits makes *A. senegal* a promising species for domestication and breeding in drylands Africa. Domestication is a man-driven process that involves selection processes rendering the target species increasingly useful to human beings by increasing the yield and/or quality of any generated product (Ræbild et al. 2010). Plant breeding is an incremental and cyclical process of selecting and crossing the most performing genotypes, and evaluating their progeny to identify the best adapted to a specific environment (Chapman et al. 2012). Thanks to the wide distribution of *A. senegal* across different environmental conditions, earlier pre-breeding programs only concerned the establishment of provenance trials in the Sahelian region of Africa. However, substantial knowledge on the adaptive potential of the species to grow under dry conditions and its performance across environments is still limited. Thus, selection of well adapted genotypes and preservation of the genetic diversity will be needed in improvement and conservation programs.

Adaptive potential and future breeding programs

With changing climate, plant populations may no longer be optimally adapted to new conditions (Anderson et al. 2012; Ikeda et al. 2017). The ability to respond to these changing gradients depends not only on the rate of environmental change (Loarie et al. 2009), but also on intraspecific genetic variability, which is critical for an evolutionary response to climate (Evans et al. 2016). In response to climate change, *A. senegal* will require high level of phenotypic plasticity or adaptive potential. Diallo et al. (2016) showed that polyploids and diploids of *A. senegal* did not differ in growth under normal conditions, while polyploids performed better than diploids when submitted to drought stress. This dominance in growth performance between polyploids and diploids can be interpreted as a result of the adaptive potential of *A. senegal* to grow under dry conditions. In addition, increased WUE is a potentially valuable trait that might be expected to be under strong selection pressure and hence highly heritable in dry tropical areas (Gray et al. 2013). The identification of improved genotypic/phenotypic WUE traits in species could increase the potential for tree improvement (Gray et al. 2013). Thus, the identification of optimal genotypes particularly when they are native legumes is a priority for dryland areas in Africa. Increased knowledge on genetic variation in adaptive traits in *A. senegal* will lead to identification of superior genotypes in future breeding programs.

Summary of material and method and results

The studies are carried out in common garden trials established in 1994 at Dahra and Bambey in Senegal. Both trials originally consisted of *A. senegal* trees from 18 populations across the natural distribution of the species in Africa and Asia (Table 1). The experiment was a randomized complete block design with four blocks. Each block was divided into 18 plots, corresponding to one population represented by 25 trees, i.e. 1800 trees at the time of establishment. Trees were spaced by 5 × 5 meters. The tested populations originate from areas with annual mean precipitation from less than 300 mm to 800 mm, and annual mean temperature ranges between 21.5 ºC and 29.9 ºC. Table 1 shows the detailed information on populations and sites used for each study.

Table 1 Origins and environmental characteristics of *A. senegal* populations used in each study (Manuscript)

Population (18)	Country	Latitude	Longitude	Altitude (masl)	Mean annual temperature (ºC)[a]	Rainfall (mm)	Manuscript 1 (16)	Manuscript 2 (15)	Manuscript 3 (16)
Diamenar	Senegal	16º00’ N	15º54’ W	20	27.13	288	×	×	×
Djigueri	Mauritania	15º44’ N	08º40’ W	226	28.92	309	×	×	×
Karofane	Niger	14º18’ N	06º11’ E	280	28.38	366	×	×	×
Sudan	Sudan	12º44’ N	29º35’ E	620	26.84	408	×	×	×
Kirane	Mali	15º23’ N	10º15’ W	140	29.32	380	×	×	×
Kankoussa	Mauritania	15º56’ N	11º27’ W	80	29.71	294	×	×	×
Chad	Chad	12º49’ N	15º18’ E	280	28.97	349	×	×	×
Daiba	Senegal	15º22’ N	13º08’ W	28	29.56	437	×	×	×
Aite	Mali	15º05’ N	11º39’ W	80	29.65	449	×	×	×
Di	Burkina Faso	13º10’ N	03º25’ W	260	28.02	637	×	×	×
Bissiga	Burkina Faso	12º26’ N	00º32’ W	280	28.28	696	×	×	×
Kidira	Senegal	14º28’ N	12º13’ W	39	28.93	611	×	×	×
Somo	Mali	13º17’ N	04º54’ W	40	27.45	627	×	×	×
Ngane	Senegal	14º08’ N	16º12’ W	2	27.92	570	×	×	×
Sodera	Ehiopia	08º24’ N	39º23’ E	1500	21.36	762	×	×	×
India60	India	29º19’ N	79º31’ E	120	25.86	867	×		×
India50	India	26º19’ N	73º07’ E	209	26.56	357			
Pakistan	Pakistan	25º40’ N	67º28’ E	370	24.61	193			
Sites									
Dahra	Senegal	15º 20’N	15º 28’ W	45m	28.1	388	×	×	×
Bambey	Senegal	14º 71’N	16º 47’ W	20m	27	482		×	

Manuscript 1: Leaf morphology and stable isotope ratios of carbon and nitrogen in *Acacia senegal* (L.) Willd. trees vary with climate at the geographic origin and ploidy level

The ability of plants to use water and nutrients efficiently is crucial in the adaptation to contrasting environments (Battipaglia et al. 2012). Leaf morphology and ploidy level can influence plants' ability to adapt to climatic conditions. *Acacia senegal* has multiple ploidy levels and grows across a geographic range of mainly dry environments. The aim of this study was to explore effects of ploidy level and climate at site of origin on leaf shape and composition of stable carbon and nitrogen isotopes of *A. senegal* from different geographical populations. A common garden trial established in 1994 in an arid region, Dahra (15º 20'N, 15º 28' W, elevation 45m) in Senegal was used for data collection. Data was collected from 225 *A. senegal* living trees representing 16 populations across the species range (Fig. 1). The ploidy level was determined based on flow cytometry from samples of shoot tips with immature leaves collected from all living trees in October 2018. Nine leaf characters were measured: petiole length, number of pinnae per leaf, number of leaflets per pinna, leaf width and length, pinnae width and length, leaflet width and length (Fig. 2i). Carbon and nitrogen stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$) and total nitrogen content were determined as described by Novak et al. (2019). The analysis were based on 130 dried and ground leaf samples from 100 diploid and 30 tetraploid trees.

The results showed the presence of three levels of ploidy, namely diploid (2n), triploid (3n) and tetraploid (4n) (Fig. 1), with a dominance of diploid individuals (84%), followed by tetraploids (13.3%) and triploids (2.7%). Seven purely diploid populations were sampled whereas two purely tetraploid populations were observed (Sodera and India60). Diploid and tetraploid individuals were coexisting in five populations (Ngane, Kidira, Kirane, Aite, and Karofane), and both diploid and triploid individuals were found in the Somo population. All three levels of ploidy occurred in the Karofane population.

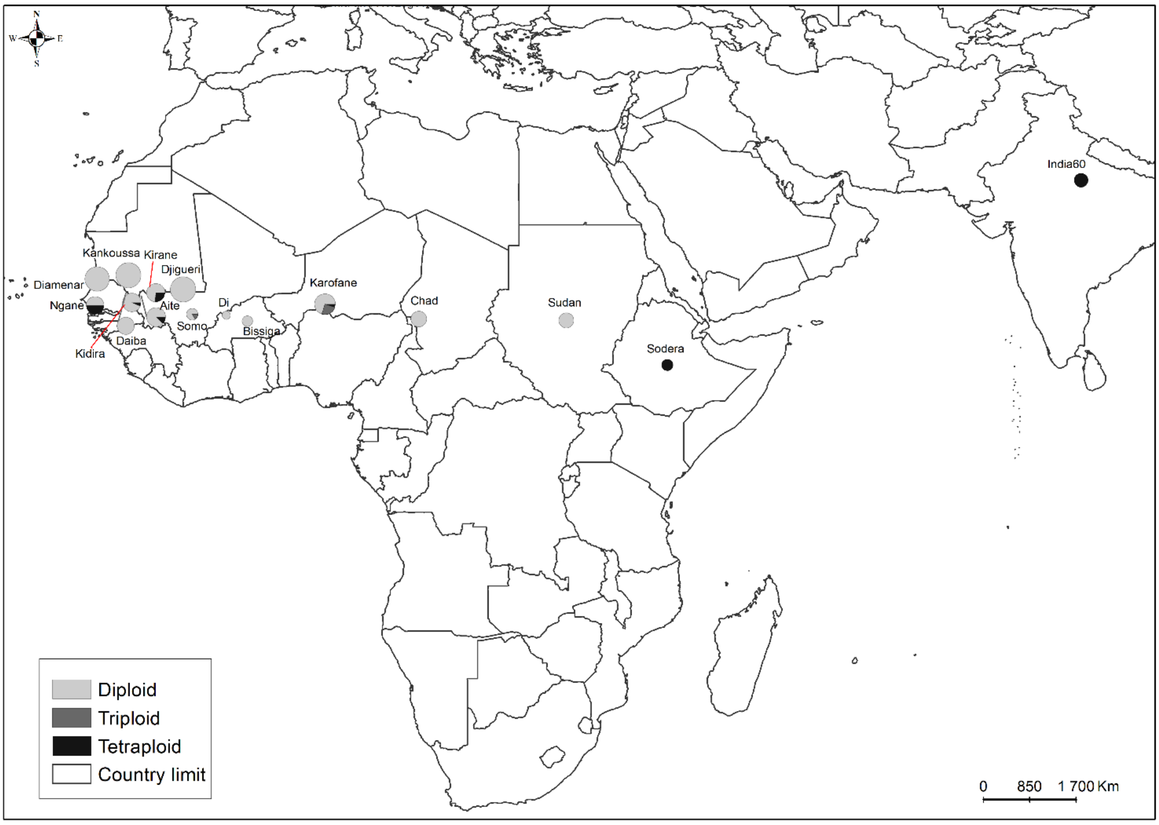

Fig. 1 Distribution of *A. senegal* ploidy levels among the 16 populations located in the common garden in Dahra, Senegal. Circle area and pie represent sample size and relative frequency.

Leaf morphology varied significantly among both geographic origins and ploidy levels, with especially triploid trees having distinct leaf shapes (Fig. 2ii).

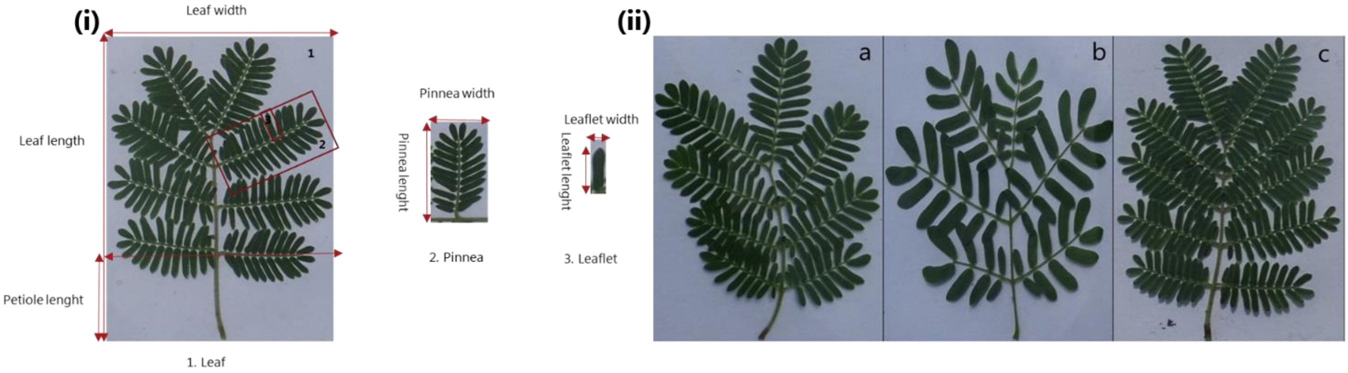

Fig. 2 Leaf morphological characters measured (i), and illustration (ii) of Diploid (a), Triploid (b) and Tetraploid (c) leaves of *Acacia senegal* from the Karofane population.

Leaf morphology, isotopic discrimination and total leaf nitrogen in *A. senegal* vary according to ploidy level and geographic origin. Tetraploids displayed high δ^{13}C, low total leaf nitrogen and low δ^{15}N values compared to diploids (Fig. 3).

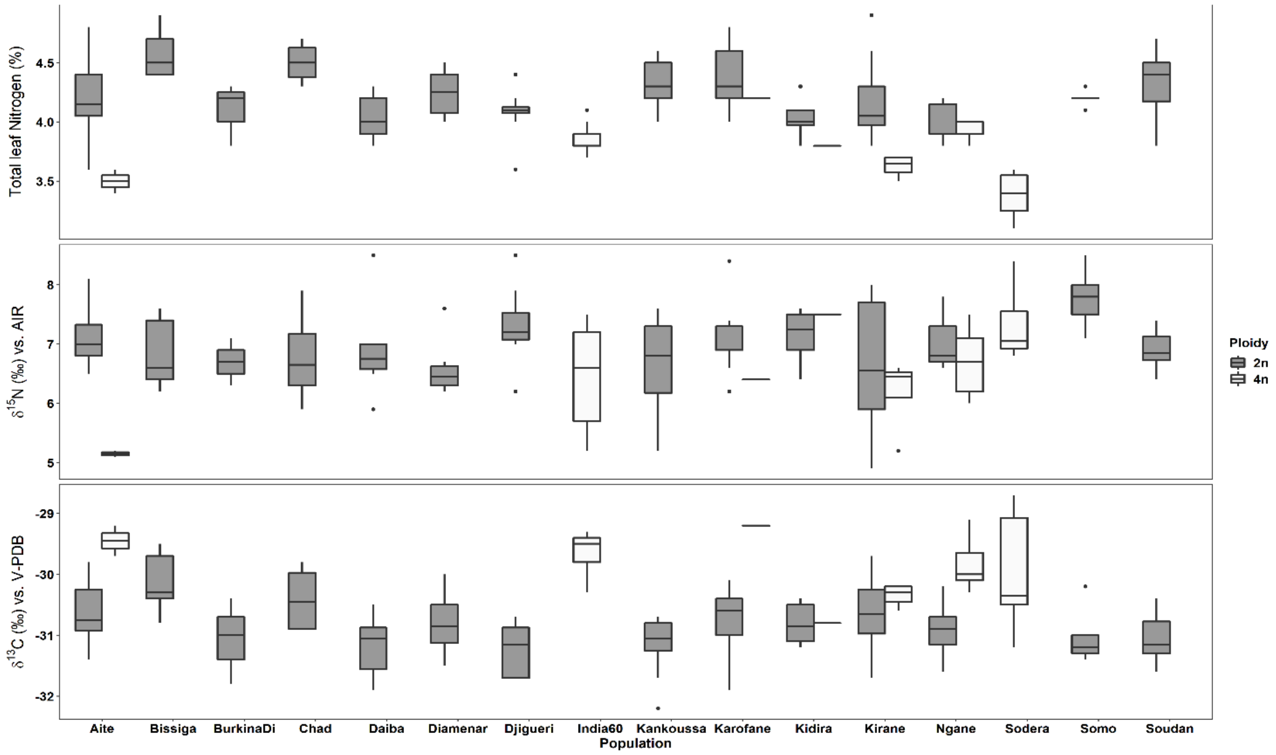

Fig. 3 Box plot of variation in carbon and nitrogen isotope ratios and total leaf nitrogen between provenance and ploidy level, lines represents provenances with only one tree at the ploidy level (n=100). Box plots represent median, first-third quartile, minimum and maximum values. Gray and white boxes represent diploid and tetraploid trees, respectively.

For diploids, leaf length and number of leaflets were correlated with precipitation and latitude, respectively (Fig. 6 Manuscript 1).

Manuscript 2: Survival and growth of *Acacia senegal* (L.) Willd. (*Senegalia senegal*) provenances depend on the rainfall at the site of origin.

Phenotypic variation is the result of the combined effect of genetic and environmental heterogeneity and their interaction (G×E interaction). The aim of this study was to investigate the survival and growth of trees from 15 African *Acacia senegal* provenances tested in two environments and to relate the differences among provenances to the climate at their site of origin and tree age. The study is based on 14 years of growth in a common garden trial at two sites (Bambey and Dahra, Senegal) that differ in water availability index (WAI), followed up by a later assessment after 23 years at one of the sites.

The present study revealed that survival, height and diameter of *A. senegal* trees varied significantly among provenances and between sites and years (Table 2). The trees in general survived better and grew faster at Bambey compared to Dahra at age 14. The interactions between provenance and year in Dahra were not significant for neither survival, nor height or diameter (Table 3, Manuscript 2).

Table 2 Least square means for survival rate, height and diameter of *Acacia senegal* provenances in Bambey and Dahra (model 1). Provenances are arranged according to precipitation.

Provenance	Survival (%)			Height(m)			Diameter (cm)		
	Bambey Age 14	Dahra Age 14	Dahra Age 23	Bambey Age 14	Dahra Age 14	Dahra Age 23	Bambey Age 14	Dahra Age 14	Dahra Age 23
Diamenar	56	36	29	4.6	3.8	4.0	10.2	7.9	13.2
Kankoussa	49	33	29	4.2	3.4	4.0	9.9	6.6	13.0
Djigueri	72	27	26	4.6	4.5	4.2	10.3	9.2	15.6
Chad	85	28	16	5.3	4.8	4.6	14.5	10.4	18.6
Karofane	69	24	22	4.3	3.7	3.9	11.0	8.4	13.2
Kirane	66	25	18	4.8	4.2	4.3	11.1	9.0	14.1
Sudan	72	44	20	4.8	4.5	4.4	11.4	9.6	13.9
Daïba	60	26	18	4.4	4.4	4.4	9.4	10.2	14.9
Aite	47	25	20	5.7	4.5	4.7	13.4	8.8	15.1
Ngane	62	21	17	5.8	4.5	4.7	15.2	9.8	16.8
Kidira	59	23	15	5.5	3.8	4.2	12.4	7.4	11.6
Somo	41	14	8	4.5	2.8	3.7	11.4	5.9	12.8
Di	30	13	3	3.9	3.8	4.1	9.9	8.6	15.6
Bissiga	62	11	9	5.2	4.3	5.0	13.3	10.0	18.9
Sodera	38	18	12	4.0	3.3	3.3	10.3	7.4	9.1
p value	0.0031**	0.0055**	0.02136*	< 0.0001***	0.1376	< 0.0001***	< 0.0001***	0.0451*	0.0003***
mean	58	25	18	4.8	4.0	4.2	11.6	8.6	14.4
S.E	11	5	5	0.2	0.5	0.2	0.5	1.0	1.2

S.E. standard error of provenance least square means

In general, trees that originated from dry sites (low rainfall and WAI0) survived better than trees from wetter sites (high rainfall and WAI0) at both test sites (Fig. 4). No relationships with climate variables were found for height and diameter.

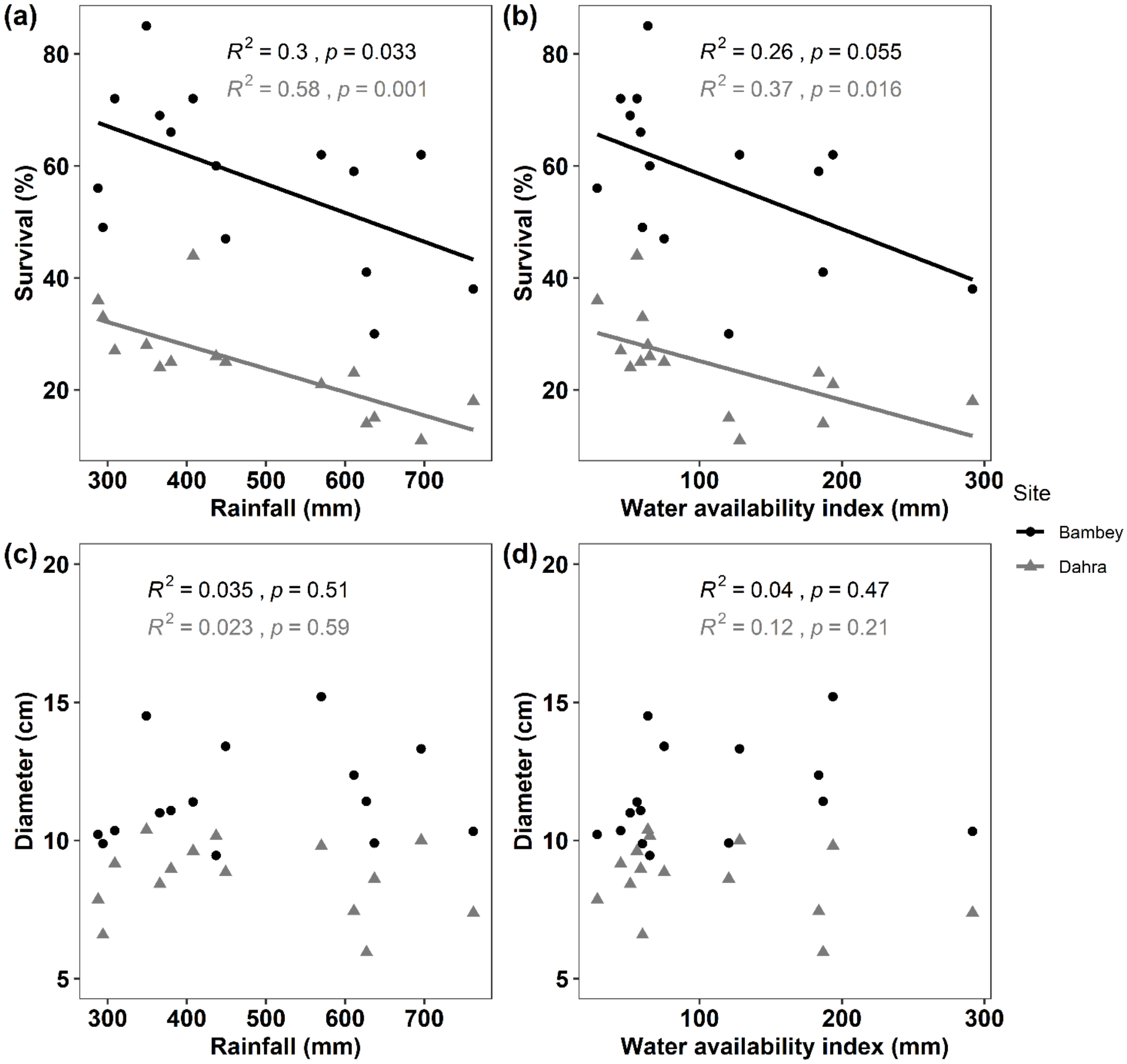

Fig. 4 Pair-wise plots of survival rate and diameter against annual rainfall (a, c) and the cumulated water availability index for the months with precipitation > potential evapotranspiration (WAI0) at Dahra and Bambey (b, d). R^2 and P-values from regression analyses are inserted.

For height and diameter, the provenances from wetter sites generally ranked better at the wet test site (Bambey) while provenances from drier sites in general ranked better at the dry site (Dahra) (Fig. 5).

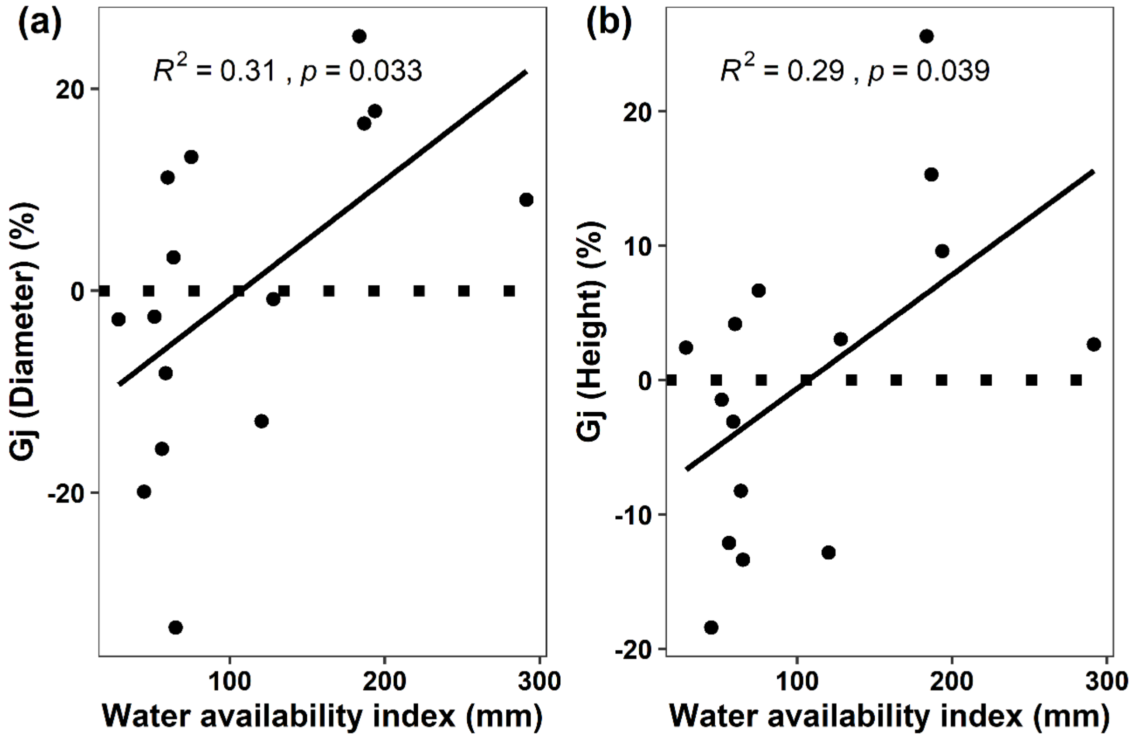

Fig. 5 The relative advantage of growth at the wetter site Bambey compared to the dry site Dahra (Gj) of the provenances related to the cumulated water availability index for the months with precipitation > potential evapotranspiration (WAI0) at the sites of origin of the provenances. Values above 0 denote relative superiority at the wet site Bambey and values below 0 denote relative superiority at the dry site Dahra.

Manuscript 3: Variation in phenology of *Acacia senegal* (L.) Willd. in relation to origin and ploidy level – implication for climatic adaptation

Heritable phenotypic variation in fitness traits is essential for species' response to environmental changes through natural selection. This can be of high relevance in dryland areas where growth is confined to the wet season. However, knowledge of genetic variation in phenology of African dryland species such as *Acacia senegal* is limited. The present study, based on the common garden trial of Dahra (cf. manuscript 1), aims at investigating variation in phenology within and among populations of *A. senegal* in Senegal. The leafing, flowering and fruiting phenology were monitored during 17 months (August 2018 to December 2019) and compared to observations of the rainfall during the same period.

Acacia senegal shed most of its leaves during the dry season and leaf development began before the rainy season. The full development of the leaf, flowering and fruiting phases occurred during the rainy season (Fig. 6).

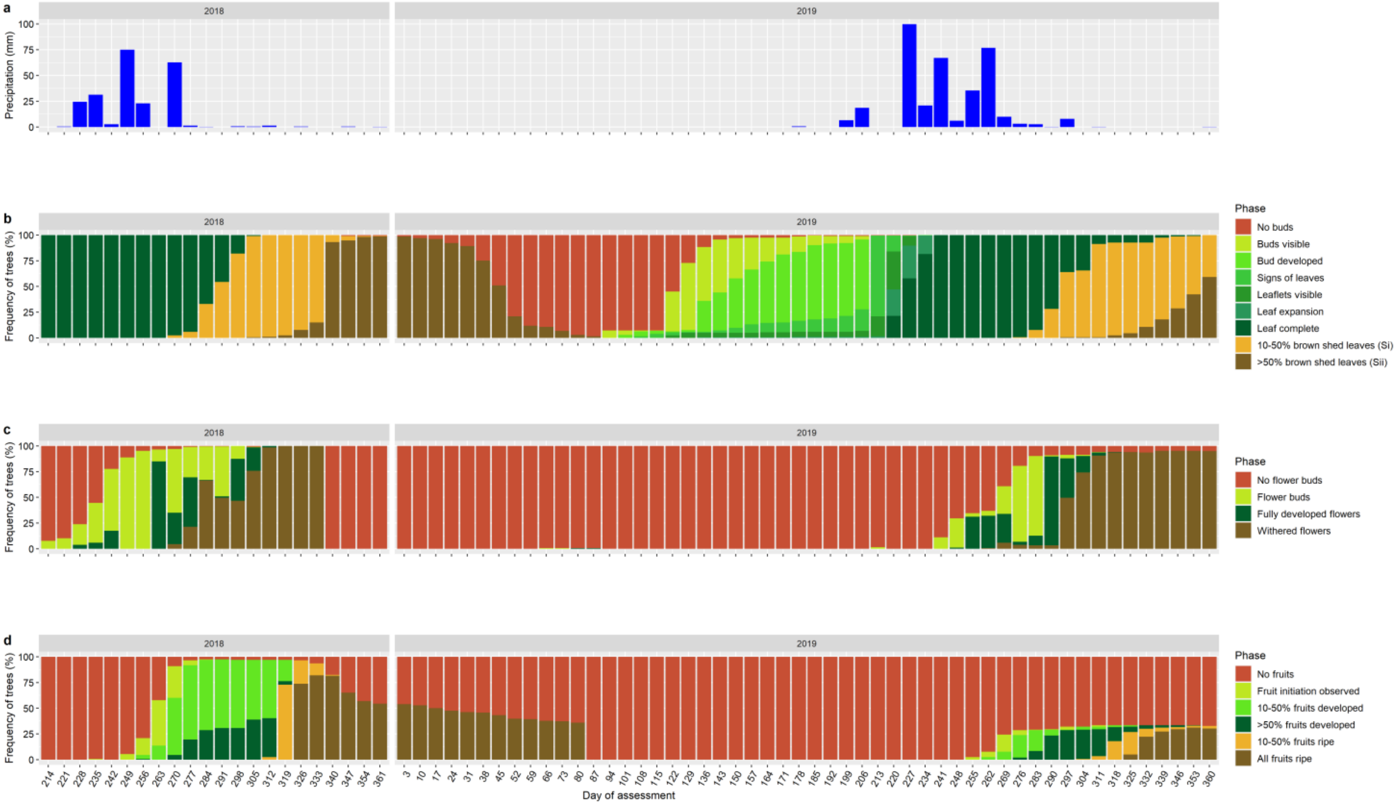

Fig. 6 Patterns of rainfall (a), leafing (b), flowering (c) and fruiting (d) phenology of *A. senegal* during the period of survey (August 2018-December 2019). Weekly accumulated rainfall (blue bars) from the weather station nearest to the Dahra field site (15°24′10″N, 15°25′56″W) in 2018 and 2019.

Phenology in *A. senegal* is under genetic control with significant differences among populations and ploidy levels. Tetraploids showed earlier signs of leaves compared to the diploids, yet, diploids had a significantly faster leaf development. Diploid populations were locally adapted to seasonality, and populations from typically dry sites had early leaf flushing and longer growing period (Fig. 7).

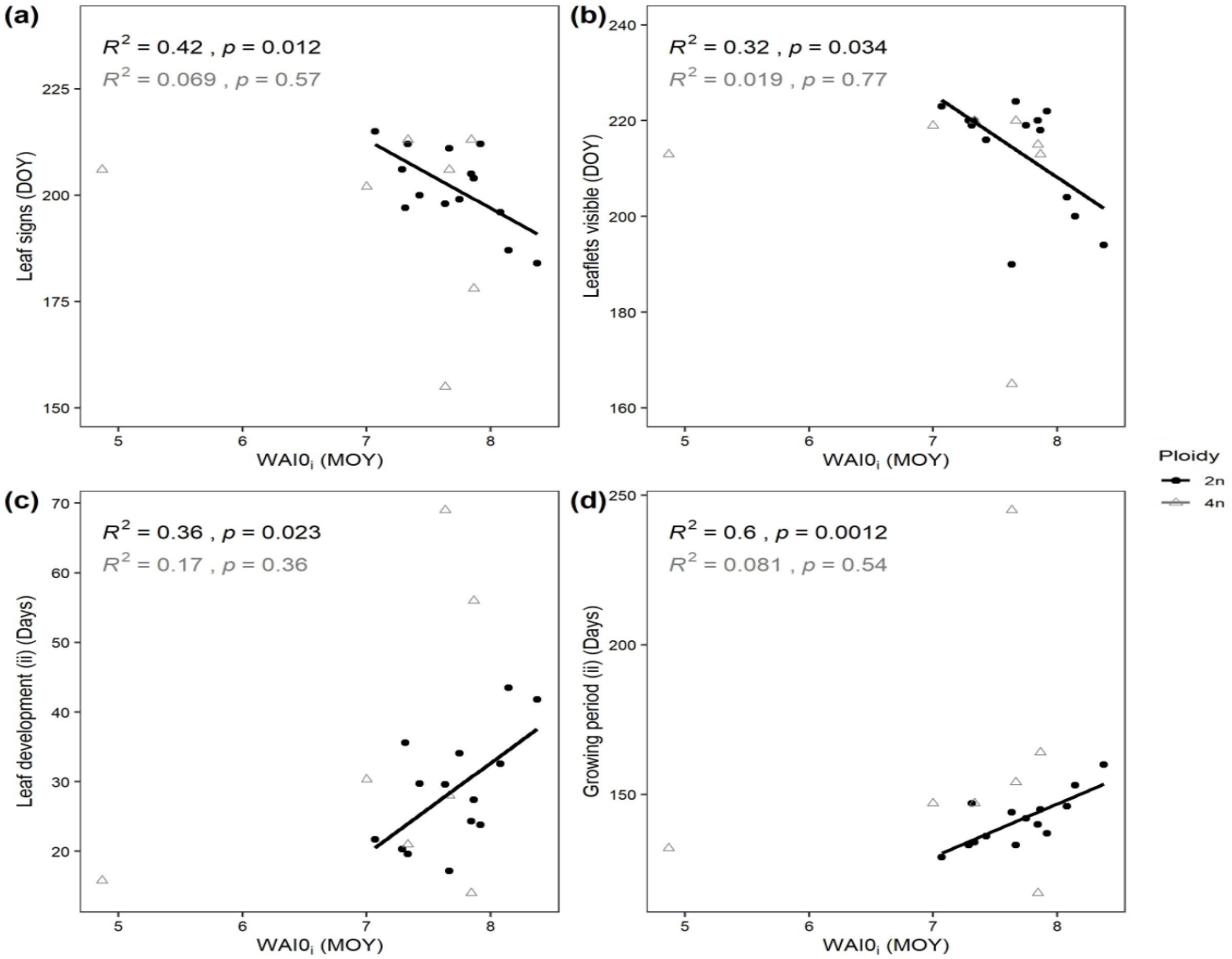

Fig. 7 Pairwise plots of the "leaf signs", "leaflets visible", "leaf development (ii)" and "growing period (ii)" against the WAI0$_i$ (first month of year (MOY) with WAI > 0 average for the period 1961-1990) (a,b,c,d), in diploid and tetraploid populations. Symbols represent population least square means. Growing period (ii) is the number of days between the first day with registration of "signs of leaves" and first day with registration of ">50% brown and shed leaves" (GP (ii)); The leaf development (ii) the number of days between the first registration of "signs of leaves" and first registration of "leaf complete" (LD(ii)).

Flowering phenology differed between diploid and tetraploid trees, but not to an extent that suggests it could provide an effective reproductive barrier between the two ploidy levels (Fig. 8).

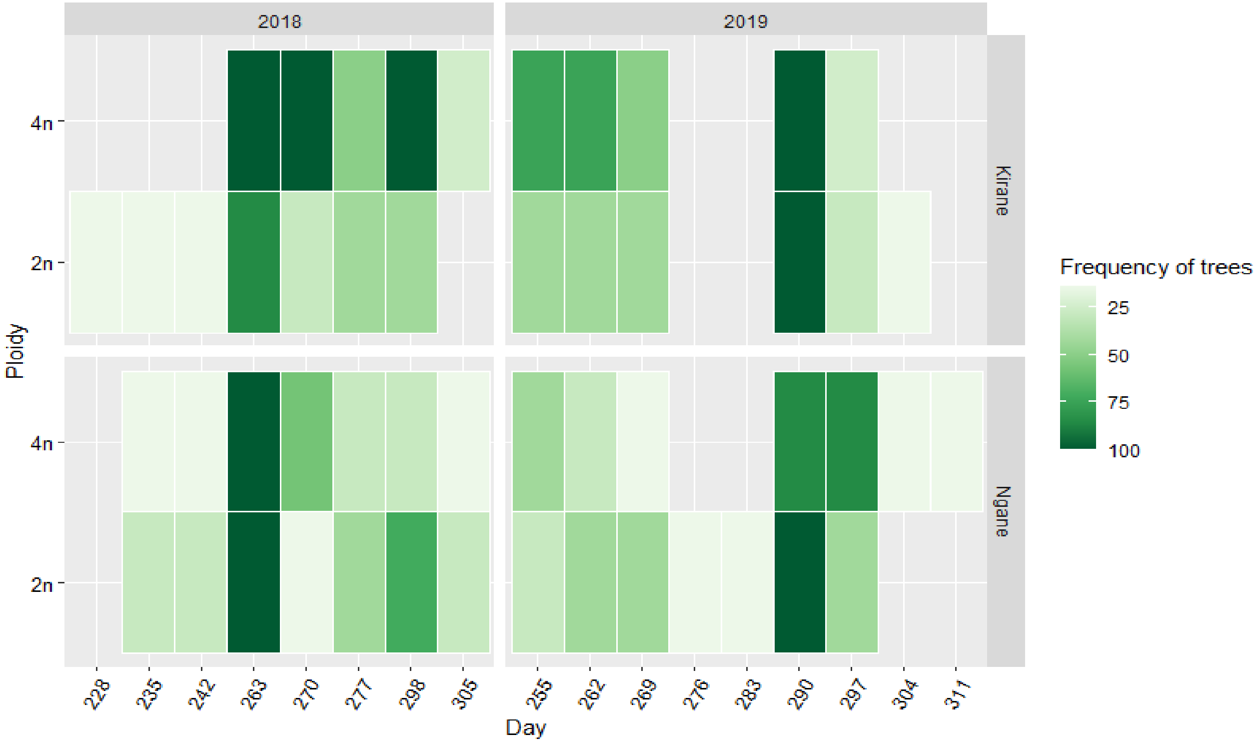

Fig. 8 Frequency of fully developed and likely fertile flowers among diploid and tetraploid groups of trees within the two populations Kirane (Mali) and Ngane (Senegal), respectively.

General discussion

Genetic variation in adaptive traits of *A. senegal*

Genetic variation among and within Sahelian tree species in their ability to grow under dry conditions represents an essential genetic resource for the species in the context of their domestication (Ræbild et al. 2010). Genetic variation among natural origins of *A. senegal* has been found in survival and growth when tested in common garden trials in Africa (Ræbild et al. 2003a,b,c; Raddad and Luukkanen 2006; Raddad 2007; Larwanou et al. 2010), and based on molecular markers (Assoumane et al. 2012, Odee et al. 2015, Diallo et al. 2015). The findings in Manuscript 1 confirm the occurrence of polyploidy in *A. senegal* with the presence of three ploidy levels namely diploid (2n), triploid (3n) and tetraploid (4n), and also that the species is mostly diploid across its natural distribution in Africa (Odee et al. 2015). Furthermore, Manuscript 1 revealed that the different ploidy levels differed in their leaf morphology with triploid individuals that were highly differentiated by larger leaves, pinnae and leaflets with fewer number of pinnae per leaf and leaflets per pinnae. However, these identified triploids resembled to the botanical description of *A. laeta* leaf morphology by Giffard et al. (1966) that gave rise to two hypotheses. Firstly, whether the triploid individuals observed have a hybrid origin between *A. senegal var. senegal* and *A. mellifera subsp. Mellifera*. Secondly, it is also possible that the triploids in our study indeed are a natural cytotype of *A. senegal* as suggested by Odee et al. (2015) where triploid individuals could have arisen either from a diploid × tetraploid cross, or from the combination of reduced and unreduced gametes of a diploid. The differences between di- and tetraploid individuals in their leaf morphology were small, i.e. tetraploids having longer leaves and petioles, which can explain why the presence of different ploidy levels in *A. senegal* were only recently recognized. However, tetraploids had the highest mean value of $\delta^{13}C$ and therefore the highest water use efficiency (WUE), but the lowest $\delta^{15}N$ and total leaf N content (Table 3 Manuscript 1) compared to diploids. These findings suggest that ploidy level influence WUE in *A. senegal* (Sarr et al. 2021), and diploids and tetraploids differ in sourcing of nitrogen. Sarr et al. (2021) reported that WUE in *A. senegal* may be impacted by others factors, since only 15% of the variation in isotopic composition was related to variation in ploidy level. This calls for further investigations to clarify the implication of poplyploidisation in adaptive traits of *A. senegal*. Still, leaf morphology vary significantly among diploid populations, while no differences were significant for the tetraploid trees. The lack of variation in leaf morphology among tetraploid populations may be a result of high rate asexual reproduction (apomixes) in tetraploids (Assoumane et al. 2012, Diallo et al. 2015).

Manuscript 3 demonstrated for the first time genetic variation in phenological traits between diploid and tetraploid individuals and among populations of *A. senegal*. Diploid and tetraploid trees differed in some leaf phenological traits ("first signs of leaves" and the speed of development of leaves). Although tetraploids developed early "signs of leaves" (day 187± 7.3 compared to the diploids at day 203 ± 5.2), diploids had a significantly faster leaf development (Table 3 Manuscript 3). For flowering and fruiting, tetraploids had "early fully developed flowers" and "flower withered" (Table 4 in Manuscript 3), but the differences in fruit development between the two cytotypes were not clear (Table 5 in Manuscript 3). In general, populations of *A. senegal* with early leaf flushing trees had a longer growing period and performed better in terms of growth at the tested site. It was mainly diploid populations from typically dry sites that had a longer growing period (Manuscript 3). This suggests different mechanisms to cope with water loss between the two cytotypes.

The differentiation in survival, height and diameter among African *A. senegal* (Manuscript 2) was found to depend on their genetic origin (Ræbild et al. 2003a,b,c; Raddad and Luukkanen 2006; Raddad 2007; Larwanou et al. 2010). In addition, genetic differentiation in growth among provenances tend to be a common feature in Sahelian tree species as found in *Faidherbia albida* (Billand and De Framond 1993), *Prosopis africana* (Weber et al. 2008; Sotelo-Montes and Weber 2009), *Balanites aegyptiaca* (Weber and Sotelo Montes 2010), *Parkia biglobosa* (Ouedraogo et al. 2012; Lompo et al. 2020), *Vachellia nilotica* (Larwanou et al. 2014), *Adansonia digitata* (Chládová et al. 2019). Manuscript 2 also documented for the first time the presence of G×E interaction in *A. senegal* for diameter with provenances from wetter sites generally ranked better at the wet test site (Bambey) while provenances from drier sites in general ranked better at the dry site (Dahra). Selection of superior provenances for planting programs must therefore be based on testing at multiple sites in order to be able to identify the best genetic origin for a specific site as proceded in many species (Wu and Matheson 2005; Correia et al. 2009; Rweyongeza 2011; Belaber et al. 2020), but our study is based on only two sites, and can therefore only be seen as a pilot study when it comes to mapping G×E.

To what extent does the variation among populations reflect local adaptation?

Previous studies have suggested presence of local adaptation in *A. senegal* when investigating common garden trials in Burkina Faso (Ræbild et al. 2003a,b,c), Niger (Larwanou et al. 2010), and Sudan (Raddad and Luukkanen 2006; Raddad 2007). However, the physiological mechanisms behind these patterns remain unknown but may reflect ecotypes based on adaptation to the climatic conditions (Larwanou et al. 2010). Local adaptation can be a result of differentiation in physiological and morphological traits as a response to divergent natural selection (Eriksson et al. 2020), or a consequence of polyploidisation (Diallo et al. 2016). Our observed genetic variations among populations in leaf morphology (Manuscript 1), survival and the presence of G×E interactions (Manuscript 2), and leafing phenology (Manuscript 3), could be interpreted as a result of local adaptation of *A. senegal* in dry conditions. Manuscript 1 suggests that variation among diploid populations in leaf characters could reflect a local adaption of *A. senegal* to dry conditions, with smaller leaves and fewer leaflets per leaf on dryer sites (see Fig. 6 Manuscript 1). This confirm that natural selection can shape differences in leaf morphology (Arntz and Delph 2001), and leaf size tend to decrease with rainfall (McDonald et al. 2003). In addition, similar patterns for number of leaflets per leaf were observed in *Parkia biglobosa* (Teklehaimanot et al. 1998) and *Adansonia digitata* (Cuni Sanchez et al. 2010; Korbo et al. 2011), suggesting a general feature of Sahelian species. The differentiation in leafing phenology in Manuscript 3 where diploid populations from typically dry sites displayed early leaf flushing and longer growing period, documents the local adaptation to seasonality in *A. senegal s*uggesting an important strategy to benefit from early rainfall and to make leaves ready to start photosynthesizing when the rainy season starts. This strengthen the findings in Manuscript 1, and we can speculate that smaller leaves and longer lived leaves in *A. senegal* could be interpreted as a mechanism of drought adaptation.

Still, Manuscript 2 indicated that *A. senegal* provenances from the drier sites of the distribution area in Africa are superior with respect to survival, thus likely reflect a local adaptation to drought.

The explanation of these resulted clines could be the differences in allocation of biomass to roots (Raddad 2007; Merine et al. 2014) as it is demonstrated that tree species adapted to dry conditions develop higher root-to-shoot ratios and deeper root systems compared to species from mesic environments (Markesteijn and Poorter 2009; Hartmann 2011; Brunner et al. 2015). In addition, the ability of plants to access soil moisture at depth are likely to influence plant survival, and the initial growth in *A. senegal* is mainly underground with seedlings developing a long tap-root (Fagg and Allison 2004). It will therefore be interesting to investigate the probability of survival among different origins of *A. senegal* by testing the rooting system of different provenances of *A. senegal* in the field and under controlled conditions. Provenances from wetter sites had superior performance with respect to height and diameter under more favorable conditions, and the patterns of G×E interactions confirm a local adaption to rainfall with respect to growth parameters (Larwanou et al. 2010; Ræbild et al. 2003a,b,c; Raddad 2007). This suggested that while survival is the most critical for fitness under dry conditions, it may come with a negative fitness trade-off against fast growth (which may increase competitiveness for other resources, and options for earlier and more abundant seed production) under more humid conditions.

Implication for the adaptive potential for use and conservation of *A. senegal*

Understanding the importance and patterns of geographic variation is significant in both tree improvement programs and gene conservation. In tree improvement programs, breeders choose provenances that are best adapted to produce the desired yield and product quality. In gene conservation programs, knowledge of geographic variation is important for designing sampling schemes to ensure that genes are conserved from all genetically distinct provenances.

A. senegal has multiple ploidy levels and grows across a geographic range of mainly dry environments. The variation in leaf morphology (Manuscript 1) and leafing phenology (Manuscript 3) in *A. senegal* reflects local adaptation, but also is an indication of differences between diploid and tetraploid individuals in their strategies to overcome drought. In diploid populations, leaf length and number of leaflets varied according to rainfall and latitudinal gradient at the site of origin, while a similar trend was not observed among tetraploid trees. Higher WUE and lower leaf N content found in tetraploids compared to diploids are characteristics for adaptation to dry conditions, suggesting that the two cytotypes may have developed different adaptation strategies to cope with harsh Sahelian growing conditions. However, the results for diploid trees indicate that the leaf phenology reflected local adaptation with early leaf flushing trees having a longer growing period and performing better in terms of growth at the tested site. This implies the complexity of the adaptive potential in the two levels of ploidy, and given the magnitude of the on-going climatic changes, more studies will be needed to address adaptation of *A. senegal* to dry growing conditions.

The results in Manuscript 2 support that divergent selection creates and maintains local adaption of *Acacia senegal* provenances in relation to growth (height and diameter) and survival in areas with different water availability. This has important implications for choice of appropriate planting material and for conservation of genetic variation among natural populations, but also for prediction of the effects of climate change. If provenances from dry sites are more successful to survive under different conditions, promoting these provenances can be of high importance for use and conservation of *A. senegal* in Senegal and Sahelian regions of Africa.

Limits of the study

The study presents some limitations that do not impede the conclusions but can provide room for future research.

i. In manuscript 1, we did not know the ploidy level of all trees at the time of establishment of the common garden trial. This limited knowledge on the distribution of polyploids across the natural range of *A. senegal*, and on their adaptive potential to perform under dry conditions. The origin of the triploid individuals needs to be clarified based on genetic markers as for instance differences in DNA sequences of the internal transcribed spacer (ITS) region, but also with comparison of the leaf morphology from previously reported triploid *Acacia senegal* trees.

ii. The studies in manuscripts 1 and 3 suggests that while the phenotypes of the diploid trees in general reflects the environment at their site of origin, this seems much less to be the case for tetraploid populations that is intriguing. It can be a result of apomixes in tetraploids that at least in theory will reduce the adaptive potential in tetraploids. However, there are fewer tetraploid trees compared to the number of diploid trees in the trials, which can influence the ability to detect any effect (power of tests). Studies based on more trees (sample size) in other species are highly recommended.

iii. In Manuscript 2 our study of G×E interaction is based on only two sites and two different ages, and can therefore only be seen as a pilot study when it comes to mapping G×E interaction and age×age correlation. A more fine-scaled set of test sites and age classes will be required to guide number and location of test sites for breeding and management of *Acacia senegal*.

Conclusion and future perspectives

The PhD thesis provides insights on the adaptive potential of *A. senegal* to grow under dry conditions. Genetic variation in adaptive traits were evaluated among populations and ploidy levels, and their implications for future breeding and conservation programs were discussed. In general, the studies show that *A. senegal* consists of provenances that are genetically differentiated in their leaf morphology, phenology and growth, and that these differences partly reflect adaptation to local climatic conditions. However, the physiological mechanisms are still not well understood, as found to be also related to the level of ploidy.

Although the differences in leaf morphology between diploids and tetraploids were small the significant variation in total leaf N and $\delta^{13}C$ implies that the two cytotypes displayed different strategies to overcome drought. In addition, the variation in $\delta^{15}N$ suggests that diploid and tetraploid individuals had different sources of nitrogen. We can conclude that the differences in leaf morphology may also be related to micromorphological features and availability of N sources, in addition to the resulted macro leaf morphology. More studies including detailed anatomical observations combined with ecophysiological tests, leaf conductance, root/shoot ratio and root architecture of diploid and tetraploid individuals, will be important in order to better address adaptation of *A. senegal* to dry growing conditions.

African provenances of *A. senegal* most likely reflect local adaptation to different ecological conditions within the species' natural distribution in Africa. The results point towards the need for development of guidelines for transfer of forest reproductive material and policies to avoid planting trees with poor adaptation in planting programs. This implies that breeding programs should carefully identify breeding and deployment zones and also highlights the importance of conserving genetic resources of the economically very valuable species based on a network of multiple populations in order to sample variation among populations. This is crucial in the face of ongoing climatic change where todays arid sites may become important seed sources for tomorrow’s planting programs in desertificated areas.

Phenology in *A. senegal* is under both genetic control and environmental influences, and populations from dry areas are likely to be more adapted. Still, the environmental cue that triggers leafing phenology, and physiological mechanisms associated with the observed patterns are unknown. Further investigations including experimentations under different temperature, photoperiodicity and water regimes will give a sight on the triggering factors in leafing. In addition, research in the field on physiological mechanisms in relation to the leaf life-span in *A. senegal* will be of high importance in understanding the strategies developed by the species to overcome drought.

Finally, our studies showed that pairs of diploids and tetraploids from the same populations are not morphologically alike and suggest a period of overlap in flowering time. Whether gene flow exists between diploid and tetraploid individuals of *A. senegal* still needs more investigation in order to clarify the presence of potential reproductive barriers. This will require additional studies on adaptive traits in combination with genomic studies.

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Manuscripts

Manuscript I. Leaf morphology and stable isotope ratios of carbon and nitrogen in *Acacia senegal* (L.) Wild trees vary with climate at the geographic origin and ploidy level

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Short title: Leaf morphology and isotopes in *Acacia senegal*

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Abstract: Leaf morphology and ploidy level can influence plants' ability to adapt to climatic conditions. Here we study *Acacia senegal* that has multiple ploidy levels and grows across a geographic range of mainly dry environments. We test if and how ploidy level and climate at site of origin influence leaf shape and ratios of stable carbon and nitrogen isotopes of *A. senegal*. The study is based on leaves collected from 225 *A. senegal* trees representing 16 populations across the species range, grown in a common garden trial in Senegal. Leaf morphological parameters were measured, and ploidy level, total leaf nitrogen (N), carbon isotope ratios ($\delta^{13}C$) and nitrogen isotope ratios ($\delta^{15}N$) were determined. Three levels of ploidy were found, namely diploid, triploid and tetraploid, but at highly different frequencies among the 16 origins. Leaf morphology varied significantly among both geographic origins and ploidy levels, with especially triploid trees having distinct leaf shapes. Tetraploids displayed high $\delta^{13}C$ and low $\delta^{15}N$ values compared to diploids. For diploids, leaf length and number of leaflets were correlated with precipitation and latitude, respectively. Leaf morphology and isotopic discrimination in *A. senegal* vary according to ploidy level and geographic origin. Our analysis suggests that the differences likely reflect adaptation to different environments, but the patterns tend to differ between diploids and tetraploids.

Keywords: Adaptation of arid zone tree species, Flow cytometry, Isotopes, Leaf morphology, *Senegalia senegal*

Declarations

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Availability of data and material: data is publicly available through the University of Copenhagen - Electronic Research Data Archive (ERDA).

Authors' contributions: OD, EDK, AMD and AR conceived the study; OD and AMD collected the data; OD, JKH, EDK and AR analysed the data; VN and KHL performed stable isotope analysis; OD wrote the first draft, which was revised with contributions from all authors.

Key message: Leaf morphology, total leaf nitrogen (N) content and carbon and nitrogen isotope ratios of *Acacia senegal* trees vary among ploidy levels and geographic origins. Leaf morphology was significantly correlated with carbon isotope composition ($\delta^{13}C$) among diploid trees, while a significant correlation was observed with nitrogen isotope composition ($\delta^{15}N$) among tetraploid trees.

1. Introduction

The ability of plants to use water and nutrients efficiently is crucial in the adaptation to contrasting environments (Battipaglia et al. 2012). Plant strategies can be reflected in differences in morphology, anatomy and physiology. Knowledge about such adaptive strategies and potentials of tree species is of increasing importance in order to ensure sustainable use and management of forest genetic resources. The Intergovernmental Panel for Climate Change (IPCC) predicts a global increase in temperature and substantial reduced precipitation in some regions (IPCC 2019). Future water deficits in plants and crops are therefore a serious concern. Sahelian ecosystems have already been severely affected by recurrent drought and human activities (including land clearing for farmland expansion, livestock herding) (Gonzalez 2001; Vincke et al. 2009; Khan et al. 2010; Brandt et al. 2014). Still, many Sahelian agroforestry tree species have natural distribution areas that cover ecological gradients from dry to very dry sites. The gradients have created genetic variation among and within populations in their ability to grow under dry conditions and this variation represents an essential genetic resource for the species also in the context of their domestication (Ræbild et al. 2010).

Polyploidy or genome duplication, leading to possession of more than two sets of chromosomes in plant species, is known to play an important role in plant evolution and ecology (Soltis et al 2014; Ramsey and Ramsey 2014; Chansler et al. 2016) and changes species' ability to thrive under harsh conditions (Maherali et al. 2009; Manzaneda et al. 2012; Diallo et al. 2015; Zhang et al. 2015; Baker et al. 2017; De Baerdemaeker et al. 2018). Polyploidisation is often associated with morphological changes such as development of thicker and larger leaves, increased number of leaf hairs, larger stomata and lower stomatal density, which may be related to an increased ecological tolerance to drought relative to the parental species (De Baerdemaeker et al. 2018). Polyploidy can also result in changes of plant hydraulics such as an increased osmotic water potential at full turgor and modifications of the water relations and gas exchange (Li et al. 1996; Maherali et al. 2009; Chansler et al. 2016; Baker et al. 2017; De Baerdemaeker et al. 2018). Polyploids are often more tolerant to water stress than their diploid relatives as observed in *Betula papyrifera* (Li et al. 1996), *Lonicera japonica* (Li et al. 2009), *Chamerion angustifolium* (Maherali et al. 2009), *A. senegal* (Diallo et al. 2015) and *Malus sp.* (Zhang et al. 2015; De Baerdemaeker et al. 2018), perhaps due to their changed anatomy.

Variation among trees in their leaf morphological traits, including stomatal density and distribution, and epidermal features is of special interest in relation to climatic adaptation, because such traits may affect gas exchange and thereby plants' responses to key environmental factors such as light, atmospheric humidity, and CO_2 levels (Xu and Zhou 2008). It is known that leaf morphology can reflect environmental conditions (Halloy and Mark 1996; Traiser et al. 2005; Royer et al. 2008; Peppe et al. 2011; Schmerler et al. 2012; Koksheeva et al. 2017), and link to drought adaptation (Abrams et al. 1990). For example, plants in dry regions tend to have small leaves compared to plants from humid areas (McDonald et al. 2003). Several studies on tree species across a range of habitats have coupled genotypic variation in morphology and physiology with drought adaptation (Abrams et al. 1990; Abrams et al. 1992; Teklehaimanot et al. 1998; Royer et al. 2008).

Under drought stress, plants can rapidly reduce stomatal opening to reduce water loss, thereby achieving a high water use efficiency (WUE) (Raddad and Luukkanen 2006). Plants show both acclimation and adaptation to soil water deficit and soil N deficit by producing thicker leaves with high leaf mass density, lower specific leaf areas and low N concentrations (Lambers et al. 2008). The composition of stable carbon isotopes as a proxy of WUE is to some extent influenced by leaf morphology (Guet et al. 2015), and genetic variations in WUE has been found to be related to the climate of the origins (Comstock and Ehleringer 1992; Anderson et al. 1996). When investigating ecotypes of *Hymenoclea salsola* in a common garden in Southwestern USA, Comstock and Ehleringer (1992) found that differences in C isotope discrimination were related to variation in temperature, rainfal and seasonal leaf-to-air water vapor of the origins. Anderson et al. (1996) found that differences in C isotope and leaf characteristics reflect genetic adaptation to native habitats among *Eucalyptus* species growing in a common garden in New South Wales. While leaf size may change the CO_2 and H_2O fluxes into and out of the leaf, due to modification of the leaf boundary layer, differences in leaf thickness will significantly affect WUE, with thinner leaves predicted to exhibit lower WUE than comparable thicker leaves (Stanhill 1986). Thicker leaves usually have a higher density of chlorophyll per unit leaf area, i.e a greater photosynthetic capacity than thinner leaves (Craufurd et al. 1999; Hultine and Marshall 2000), and as a response to dry conditions, a reduction of leaf area and transpiration will lead to increasing WUE (Lu et al. 1997; Craufurd et al. 1999).

The role of N_2-fixation is of particular importance for leguminous tree species under nutrient limited conditions, but can change over time (Isaac et al. 2011). Experiments with legumes (*Acacia spp.*) in glasshouse and field have revealed that seedlings capture N from fixation, while mature trees to a larger extent obtain N from soil organic matter mineralization (Cramer et al. 2007). Stable isotope ratios of N ($\delta^{15}N$) in plants have been used as an indirect measure of N cycling parameters (Craine et al. 2015), and ^{15}N natural abundance has been used to estimate N_2 fixation in leguminous trees growing naturally or in agroforestry systems (Raddad et al. 2005). The $\delta^{15}N$ source is preserved during N absorption, assimilation and translocation, and leaf $\delta^{15}N$ reflects the N source in the soil (Evans 2001). Leguminous species obtain their N from the soil and through symbiotic fixation (Raddad et al. 2005), and species of *Acacia* are well known for their ability to access deep water resources (Otieno et al. 2005), and to fix N under natural conditions.

Acacia senegal (L.) Willd. (syn. *Senegalia senegal* (L.) Britton) is a leguminous tree widely distributed in Sudano-Sahelian zones in Africa, India and Pakistan. It usually occurs in sandy soils and is well adapted to extreme rainfall and temperature conditions. In the Sahel, the species occurs naturally on sites with 100-400 mm of annual rainfall and temperatures of 20-36°C (Fagg and Allison 2004). Four morphological varieties have been identified: *senegal, kerensis, rostrata* and *leiorhachis* (Fagg and Allison 2004). The variety *senegal* is the main gum arabic producing tree species in Sudano-Sahelian regions (Fagg and Allison 2004; Raddad and Luukkanen 2006; Diallo 2015). *Acacia senegal* is also a source of nutrition for animals, it restores soil fertility by N fixation, and is overall an important component of traditional agroforestry systems in tropical drylands (Fagg and Allison 2004; Raddad and Luukkanen 2006; Omondi et al. 2018).

Previous studies, unaware of the different ploidy levels within the species, have shown genetic variation in survival and growth between different origins of *A. senegal* (Raebild et al. 2003a,b,c). Larwanou et al. (2010), based on a common garden trial in Niger, found a tendency to geographic and ecological clines where the survival of the origins was correlated to the precipitation and latitude of the origin, whereas growth was mainly correlated to latitude, longitude and altitude at the place of origin. The physiological mechanisms behind these patterns remain unknown but may reflect ecotypes based on adaptation to the climatic conditions (Larwanou et al. 2010). Investigations in carbon isotope ratios revealed genetic variation among populations from clayey and sandy sites when growing at a clayey site in Sudan (Raddad and Luukkanen 2006), and among populations from the distribution area of the species in a common garden trial in Senegal (Gray et al. 2013). These variations indicate that different WUE strategies related to growth and gum production of the populations, and to environmental conditions of the origins. Earlier studies on a range of *Acacia* species showed that carbon isotope ratios were closely correlated to intrinsic water use efficiency, and, if African *Acacia*'s were considered alone, to the whole-plant transpiration efficiency (Konate et al. 2016).

In 2012, it was shown that *A. senegal* included both diploid and tetraploid individuals (Assoumane et al. 2012). Later studies revealed the presence of triploid, tetraploid, pentaploid and hexaploid individuals of the species across its natural range in Africa, India and Pakistan (Odee et al. 2015; Diallo et al. 2015; Diallo et al. 2016). Polyploidisation in *A. senegal* has been suggested to play an important role in the species' ability to thrive under dry conditions particularly in the arid regions in Africa where droughts are recurrent. Diallo et al. (2016) showed that polyploid seedlings grew faster than diploids under drought-stress conditions, suggesting that the existence of different ploidy levels in *A. senegal* can increase its potential to adapt to future climatic fluctuations in Africa. Recently, Sarr et al. (2021) found that diploid and tetraploid populations differed in their foliar isotope ratios of carbon in a progeny trial in Senegal. However, only 15% of the variation in isotope composition was explained by the ploidy level, which suggests the existence of other factors affecting WUE in *A. senegal*.

No previous studies have investigated whether *A. senegal* leaf morphology is related to climate at their site of origin or the ploidy level. In the present study, we hypothesized that variation in leaf morphology in *A. senegal* reflects adaptation to the place of origin and/or is related to the level of ploidy. We tested our hypothesis by first investigating if variation in nine leaf traits were reflecting ploidy levels in *A. senegal,* and secondly if variation in leaf morphology and ploidy were correlated with water use efficiency, total leaf N content and the C and N isotope ratios (δ^{13}C and δ^{15}N). We also compared the leaf morphological variation to the climatic conditions at the place of geographic origin.

2. Materials and methods

2.1. The common garden experiment

We studied *A. senegal* trees in a common garden (provenance) trial in Senegal, established in August 1994 in Dahra (15º 20'N, 15º 28' W, elevation 45m). The soil in the trial was a sandy (85.3%) and reddish arenosol with a pH of 6.05, a total carbon content of 0.52%, and a nitrogen content of 0.05% (Ndoye et al. 2012). The natural vegetation consisted mainly of grass and sparse trees such as *Acacia tortilis* subsp. *radiana*, *A. senegal* and *Balanites aegyptiaca* (Göttsche et al. 2016). The site represented very dry growing conditions with a mean annual rainfall of 402 mm and annual mean temperature of 28.1 ºC (WorldClim 2 2017).

The trial was established with *A. senegal* var. *senegal* trees from 18 populations across the natural distribution of the species in Africa and Asia. The experiment was a randomized complete block design with four blocks. Each block was divided into 18 plots, corresponding to one population represented by 25 trees, i.e. 1800 trees at the time of establishment. Trees were spaced by 5 × 5 meters. The site was uniform with no obvious topographic variation within the trial. The tested populations originate from areas with annual mean precipitation from less than 300 mm to 700 mm, and annual mean temperature ranges between 21.5 ºC and 29.9 ºC (Table 1).

The tree mortality from 1994 - 2018 was high, and at the time of evaluation in August 2018, only 16 populations (Table 1) with a total of 225 surviving trees (out of the originally 1800 planted trees) were left to form the basis on the present study.

Table 1 Origins and environmental characteristics of *A. senegal* populations used in the study

Population	Country	Latitude	Longitude	Altitude (masl)	Mean annual temperature (ºC)[*]	Annual precipitation (mm)[*]	Aridity Index
Diamenar	Senegal	16º00’ N	15º54’ W	20	27.13	288	8
Kankoussa	Mauritania	15º56’ N	11º27’ W	80	29.71	294	7
Djigueri	Mauritania	15º44’ N	08º40’ W	226	28.92	309	8
Chad	Chad	12º49’ N	15º18’ E	280	28.97	349	9
Karofane	Niger	14º18’ N	06º11’ E	280	28.38	366	10
Kirane	Mali	15º23’ N	10º15’ W	140	29.32	380	10
Sudan	Sudan	12º44’ N	29º35’ E	620	26.84	408	11
Daiba	Senegal	15º22’ N	13º08’ W	28	29.56	437	11
Aite	Mali	15º05’ N	11º39’ W	80	29.65	449	11
Ngane	Senegal	14º08’ N	16º12’ W	2	27.92	570	15
Kidira	Senegal	14º28’ N	12º13’ W	39	28.93	611	16
Somo	Mali	13º17’ N	04º54’ W	40	27.45	627	17
Di	Burkina Faso	13º10’ N	03º25’ W	260	28.02	637	17
Bissiga	Burkina Faso	12º26’ N	00º32’ W	280	28.28	696	18
Sodera	Ehiopia	08º24’ N	39º23’ E	1500	21.36	762	24
India60	India	26º19’ N	79º31’ E	120	25.86	867	24

[*] Climate data derived from WorldClim-Global Data Version 2.0 ((Fick and R.J., 2017) based on coordinates of the populations. Martonne Aridity index calculated as P/(T + 20) where P is annual precipitation and T is mean annual temperature.

2.2. Determination of the ploidy level

In October 2018, samples of shoot tips with immature leaves from 225 living trees were collected and conserved at 8ºC until shipment for Plant Cytometry Services, Netherlands. Here, ploidy was determined based on flow cytometry following the method described by Arumuganathan and Earle (1991). Fresh leaf material of the samples was chopped together with leaf material of the internal standards (*Vinca major* and *Ophiopogon planiscapus* 'Niger'). DAPI is a fluorescent dye, which selectively complexes with double-stranded DNA to give a product that fluoresce at 465 nm. DAPI has specific DNA-binding properties with preference for adenine-thymine (AT)-rich sequences. After chopping, the buffer (ca. 2 ml), containing cell constituents and large tissue remnants, was passed through a nylon filter of 50 µm mesh size. After 30-60 minutes of incubation of the nuclei, the samples were analysed with a Sysmex CyFlow Ploidy analyser with a UV High power LED (365) and Objective 40 x N.A. 0.8 air (Partec).

2.3. Leaf sampling and measurement

Leaf morphology was assessed on all living trees in the trial from 26th September-3rd October 2018. From each tree, three asymptomatic sun-exposed leaves of three randomly selected branches were collected, placed next to a ruler and immediately photographed with a camera before leaves started curling. Nine leaf characters were later manually measured based on the pictures. *Acacia senegal* has bipinnate leaves, and for each leaf one pinna was randomly chosen, and for each pinna three random leaflets were chosen from the middle. The following characters were then measured: petiole length, number of pinnae per leaf, number of leaflets per pinna, leaf width and length, pinnae width and length, leaflet width and length (Fig. 1).

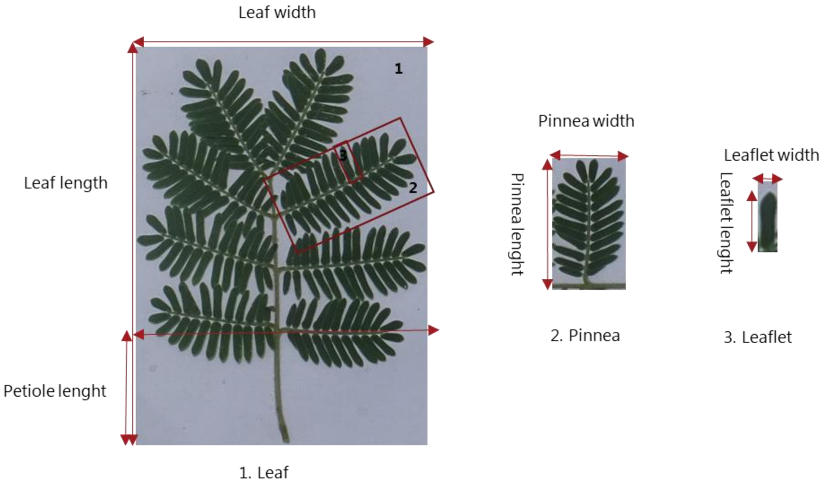

Fig. 1 *Acacia senegal* leaf morphological characters measured

2.4. Carbon and nitrogen stable isotope ratio analysis and nitrogen content quantification

In order to test for variation in total leaf N, $\delta^{13}C$ and $\delta^{15}N$ and their relationship to leaf morphology, ploidy and place of origin, several completely healthy-looking leaves were randomly collected around the canopy of each living tree, immediately air dried and stored in paper bags. In total, 130 leaf samples were collected from 100 diploid and 30 tetraploid trees. Before analysis, samples were dried at 60ºC for 48 h and grinded with a ball mill. The stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) were determined as described by Novak et al. (2019) with a PYRO Cube Elemental Analyser (EA) (Elementar, Hanau, Germany) coupled to an Isoprime100 mass spectrometer (Elementar, Manchester, UK). An amount of 4-5 mg of leaf powder was transferred into a tin capsule and analyzed in the EA combustion mode. Raw isotope data ($\delta^{13}C$, $\delta^{15}N$) were corrected for drift using a nylon intra-laboratory standard (obtained from the Stable Isotope Facility at UC Davis, CA, USA) at every 13th position, linearity using six samples of acetanilide (Sigma-Aldrich) in decreasing amount at the start and end of the sequence, and two-point calibrated using United States Geological Survey L-glutamic acid reference isotope standards USGS 40 and USGS 41 (purchased from the International Atomic Energy Agency, Vienna, Austria). The precision, quantified as one SD from the average nylon δ-value, was < 0.2‰ and < 0.1‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively.

Isotope values were reported using conventional δ-notation according to the following equation:

$$\delta^{h}\mathrm{E} = \frac{R_{\mathrm{sample}} - R_{\mathrm{standard}}}{R_{\mathrm{standard}}} \qquad (1)$$

where h is the heavy isotope of an element E (e.g. ^{13}C or ^{15}N) and R represents the corresponding isotope ratio of heavy/light isotopes (e.g. $^{13}C/^{12}C$ or $^{15}N/^{14}N$) of the sample (R_{sample}) and standard (R_{standard}). The δ-notation isotope values were reported in parts per thousand (‰) with respect to the international standards (R_{standard}): Vienna Pee Dee Belemnite (V-PDB) for $\delta^{13}C$, and AIR for $\delta^{15}N$.

The foliar N content was calculated from the stable isotope data using the acetanilide (Sigma Aldrich) calibration curve. The analytical error was determined as one SD of repeatedly measured N content in samples of acetanilide and was 0.2%.

Here, we used the stable isotopes ratio ($^{13}C/^{12}C$) as a proxy for WUE, and ($^{15}N/^{14}N$) to explore the plant N source.

The plant $\delta^{13}C$ values ($\delta^{13}C_{\mathrm{plant}}$) are controlled by $\delta^{13}C$ values of atmospheric CO_2 ($\delta^{13}C_{\mathrm{air}}$) at -8 ‰ (Graven et al. 2017) and photosynthetic discrimination ($\Delta^{13}C$) against $^{13}CO_2$ during assimilation according to equation 2 (Farquhar and Richards 1984):

$$\Delta^{13}\mathrm{C} = \frac{(\delta^{13}\mathrm{C}_{\mathrm{air}} - \delta^{13}\mathrm{C}_{\mathrm{plant}})}{\left(1 + \delta^{13}\mathrm{C}_{\mathrm{plant}}\right)} \qquad (2)$$

The C isotope discrimination ($\Delta^{13}C$) occurs during the diffusion of CO_2, and during the carboxylation by Rubisco. Therefore, plant $\delta^{13}C$ values are controlled by the intercellular CO_2 concentration that derived from the CO_2 consumption during assimilation and CO_2 supply by stomatal conductance. $\delta^{13}C$ is a proxy for iWUE (Farquhar et al. 1982, Seibt et al. 2008, Graven et al. 2017), where increasing plant $\delta^{13}C$ values reflect increasing iWUE (Farquhar et al. 1982).

2.5. Data analysis

Differences between origins and ploidy levels were tested using the R statistical package software (R Core Team 2020), and figures were produced using the package *ggplot2* (Wickham 2016).

First, Fisher's Exact Test of Independence was applied to test whether the frequency of *A. senegal* trees with different levels of ploidy was different among populations.

Leaves from the triploid trees (six trees in total) were highly differentiated from the diploid and tetraploid ones (see Fig. 3b). This led us to the concern that triploid trees may represent the fertile hybrid *A. laeta* between *A. senegal* and *A. mellifera* (see discussion), and we therefore removed triploid trees data from the analyses.

Secondly, the differences between traits in relation to population origin and ploidy level were analyzed for each of the nine morphological leaf characters, total leaf N, $\delta^{13}C$ and $\delta^{15}N$. Average values of the three leaves per tree were calculated for each of the nine characters. A spatial analysis of variables revealed no spatial structure within the trial allowing us to analyse the data based on single tree observations. For each trait, the significance of a spatial first-order autoregressive correlation matrix for ordered spatial coordinates in the field trial (rows or columns) was tested by application of the program ASReml and by use of loglikelihood ratio tests (Gilmour et al. 2015). The results are summarized in table S1 (supplementary material).

As the frequency of polyploid individuals differed highly among populations (from 0% to 100% of trees; cf. results below), effects of geographic origin and ploidy level were confounded. We therefore used a two steps approach. First we analysed the effect of ploidy for only the five populations where both diploid and tetraploid trees were present, based on the general linear model (model 3):

$$\mathbf{Y}_{ijk} = \boldsymbol{\mu} + \mathbf{B}_i + \mathbf{G}_j + \mathbf{P}_k + \boldsymbol{\varepsilon}_{ijk}, \qquad \mathbf{(3)}$$

where Y_{ijk} is the analysed trait of tree *ijk*, μ is the general mean, B_i is the random effect of block *i*, G_j is the fixed effect of geographic origin *j*, P_k is the fixed effect of ploidy level *k* and ε_{ijk} represent the residual errors assumed to be independent and normal distributed $(0, \sigma^2)$.

The second step was to analyze the variation among origins. Here, we made the analysis separately for diploid and polyploid trees based on the following model to avoid confounding effects of origin and ploidy (model 4):

$$\mathbf{Y}_{ij} = \boldsymbol{\mu} + \mathbf{B}_i + \mathbf{G}_j + \boldsymbol{\varepsilon}_{ij}, \qquad \mathbf{(4)}$$

with abbreviation of effects as above. Here, we thus made two analyses for each trait, one for the diploid trees and one for the tetraploid trees.

The function *lmer* (Linear Mixed-Effect Models) in the package *lme4* (Bates et al. 2015) for R (R Core Team 2020) was applied, and using the Kenward-Roger method adjusted degrees of freedom. Residuals were plotted against predicted values to control for variance homogeneity and residual frequency histograms were used to check for outliers and a reasonable distribution close to normal. Two trees, Karofane1 in block 4 and Djigueri5 in block 1 were detected as distinct outliers based on their leaf characters (details on students residuals in Table S2). These two observations were therefore removed from the entire analysis.

Pearson's correlation coefficients were calculated to test whether leaf morphology, total leaf N, $\delta^{13}C$ and $\delta^{15}N$ values, and geographic and climatic parameters were associated (Table 1). These tests were based on the population means estimated for diploids and tetraploids separately using model 4. Analysis were performed using the *corr.test* function in the package *psych* (Revelle 2019) implemented in R (R Core Team 2020).

Multivariate analyses were applied to study the variation in leaf morphology. We used both an un-informed and informed approach. For the un-informed approach, we performed Principal Component Analysis (PCA) as implemented in the package *factoextra* (Kassambara and Mundt 2020) in R (R Core Team 2020). Here, the objective was to visualize any patterns in the differences among the trees that was not observed by looking at the traits individually. For the informed approach, we applied Canonical variate analysis as implemented in PROC GLM (under the MANOVA option) in the SAS® software (SAS Institute Inc., Cary, NC 2004) based on a hypothesis of difference between ploidy levels. In this analysis, the objective was to test if the combined analysis of the leaf traits could effectively discriminate between diploid and tetraploid trees. We applied Wilks' Lambda statistics as a multivariate test of the difference. Finally, we calculated the Euclidean distances between population means and used this distance matrix to compare distances between the mean of di- and tetraploid trees within each population, with distances between random sets of di- and tetraploid population means. The objective here was to test if diploid trees from a given population were more similar to tetraploid trees in their own population compared to tetraploid trees from other populations using the *ggscatter* function in the package *ggpubr* (Kassambara 2020) as implemented in R.

3. Results

3.1. Variation among populations in ploidy

The frequency of trees with different ploidy levels varied significantly among populations ($p<0.001$). Three levels of ploidy were found in the trial. Most common were diploid individuals representing 84% of the tested trees and found in 14 out of 16 populations, followed by tetraploid trees (13.3%) only found in seven populations, and triploids that had the smallest frequency (2.7%) and were observed in two populations only (Fig. 2 and Table 2). Seven purely diploid populations were sampled whereas two purely tetraploid populations were observed (Sodera and India60). Diploid and tetraploid individuals were coexisting in five populations (Ngane, Kidira, Kirane, Aite, and Karofane), and both diploid and triploid individuals were found in the Somo population. All three levels of ploidy occurred in the Karofane population. The highly uneven frequency of polyploids in the inspected populations means that effects of origin (population) and ploidy are partly confounded.

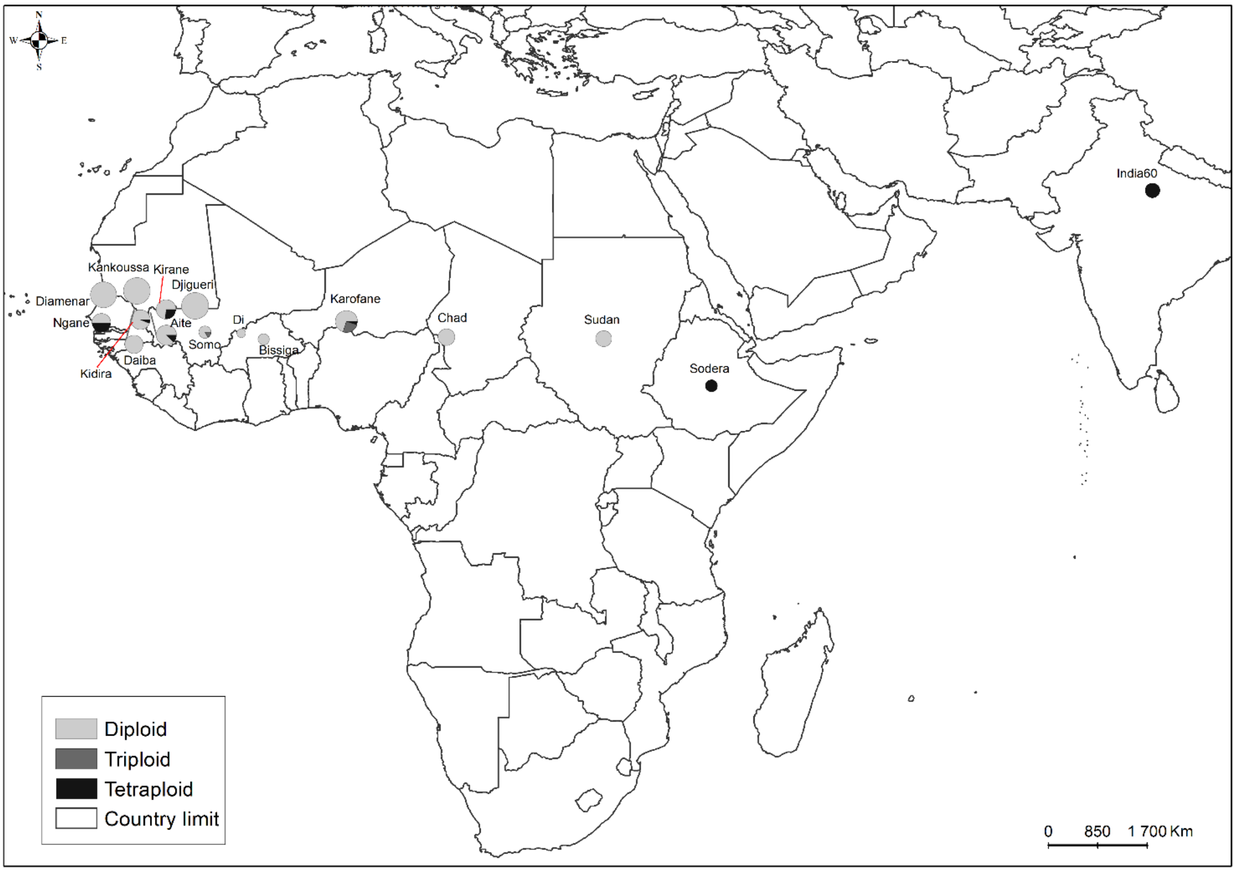

Fig. 2 Distribution of *A. senegal* ploidy levels among the 16 populations located in the common garden in Dahra, Senegal. Circle area and pie represent sample size and relative frequency of ploidy levels.

Table 2 Distribution of surviving trees among populations and ploidy levels

Provenance	Diploid (2n)	Triploid (3n)	Tetraploid (4n)	Total
Diamenar	26	0	0	26
Kankoussa	28	0	0	28
Djigueri	28	0	0	28
Chad	11	0	0	11
Karofane	14	5	1	20
Kirane	11	0	4	15
Sudan	10	0	0	10
Daiba	13	0	0	13
Aite	14	0	2	16
Ngane	7	0	7	14
Kidira	14	0	1	15
Somo	5	1	0	6
Di	3	0	0	3
Bissiga	5	0	0	5
Sodera	0	0	6	6
India60	0	0	9	9
Total	189	6	30	225

3.2. Variation of leaf traits among ploidy levels and populations

The triploid individuals were highly differentiated from the diploids and tetraploids by their larger leaves, pinnae and leaflets and by fewer numbers of pinnae per leaf and leaflets per pinnae (Fig. 3). As mentioned above, the triploids (6 individuals) were removed from the analysis as they could represent hybrids between *A. senegal* and *A. laeta*. When comparing diploids and tetraploids, significant differences were found at the ploidy level for the petiole length and leaf length with tetraploids having longer petioles and leaves (Table 3). Effects of blocks were not significant for any of the traits (Table S3).

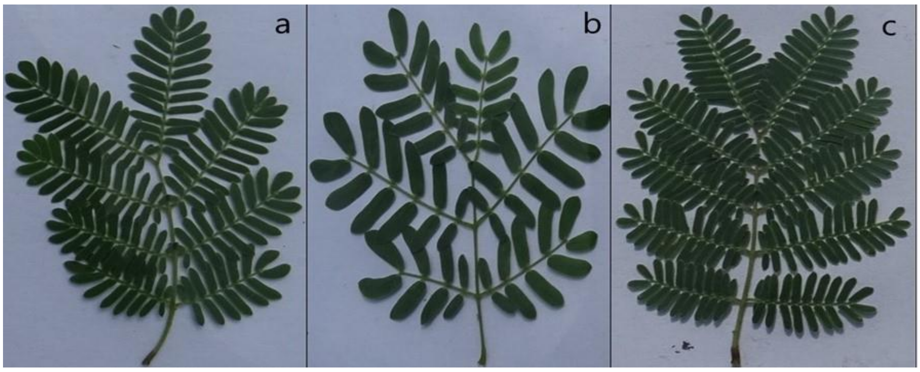

Fig. 3 Illustration of Diploid (a), Triploid (b) and Tetraploid (c) leaves of *Acacia senegal* from the Karofane population

Diploid populations were significantly different from one another in several leaf characters (number of pinnae, number of leaflets, leaflet length, leaflet width and pinnae width) while tetraploid populations did not show significant differences in any leaf traits (Table 3). No significant effect of blocks were found (Table S4).

Table 3 Results from the analysis on leaf morphology, $\delta^{13}C$ and $\delta^{15}N$ of *Acacia senegal* between diploid and tetraploid trees of *A. senegal* based on data from populations represented by diploid and tetraploid trees *(model 3)* and among diploid and tetraploid populations *(model 4)*.

Variables	Mean values				Statistical results								
					Ploidy levels (*model 3*)			Population tests (*model 4*)					
								Diploids			Tetraploids		
	Diploid	n	Tetraploid	n	Df	F value	P value	Df	F value	P value	Df	F value	P value
Petiole length (cm)	1.33 ± 0.32	59	1.56 ±0.26	15	1	9.35	**0.00324****	13	0.88	0.5838	6	0.83	0.5943
Leaf length (cm)	5.70 ± 0.73	59	6.32 ± 0.49	15	1	7.34	**0.00868****	13	1.61	0.1336	6	2.37	0.1873
Leaf width (cm)	4.18 ± 0.53	59	4.31 ± 0.44	15	1	0.42	0.7433	13	1.71	0.1064	6	1.83	0.2678
Pinnea length (cm)	2.42 ± 0.30	59	2.41 ± 0.25	15	1	0.13	0.7213	13	1.14	0.3655	6	1.02	0.5028
Pinnea width (cm)	1.12 ± 0.14	59	1.15 ± 0.10	15	1	0.05	0.82926	13	3.69	**0.00157****	6	0.74	0.6424
Leaflet length (cm)	0.57 ± 0.07	59	0.57 ± 0.06	15	1	0.12	0.73079	13	4.14	**0.00052*****	6	0.97	0.5278
Leaflet width (cm)	0.14 ± 0.02	59	0.13 ± 0.02	15	1	0.80	0.5191	13	4.00	**0.00094*****	6	3.54	0.0991
No. pinnae	6.85 ± 1.33	59	7.38 ± 1.79	15	1	1.10	0.2984	13	2.33	**0.02603***	6	0.81	0.6031
No. leaflets	24.3 ± 2.61	59	25.6 ± 2.25	15	1	3.74	0.05763	13	2.82	**0.00871****	6	1.96	0.2441
Total N (%)	4.2 ± 0.31	40	3.8 ± 0.22	15	1	12.85	**0.0008*****	13	3.21	**0.00380****	6	10.42	**0.01223***
$\delta^{15}N$ ‰	7.0 ± 0.69	40	6.4 ± 0.77	15	1	5.99	**0.01832***	13	1.47	0.1885	6	3.17	0.08625
$\delta^{13}C$ ‰	-30.7 ± 0.49	40	-29.9 ± 0.53	15	1	22.77	**< 0.0001*****	13	2.42	**0.02392***	6	0.77	0.6197

Mean ±SD of the distribution and standard deviation, Df: degrees of freedom. Asterisks indicate the level of significance; **p*<0.05; ***p*<0.01; ****p*<0.001

3.3. Variation of δ^{13}C, δ^{15}N and total leaf N

Diploids and tetraploids displayed highly significant differences in δ^{13}C and δ^{15}N and total leaf N. Tetraploids, which differed from diploids by having longer petioles and leaves, had the highest mean value of δ^{13}C and therefore the highest water use efficiency, and the lowest δ^{15}N and total leaf N content (Table 3). The effect was seen clearly when comparing diploid and tetraploid trees in the three populations that harbored both levels (Kirane, Aite and Ngane, Fig. 4).

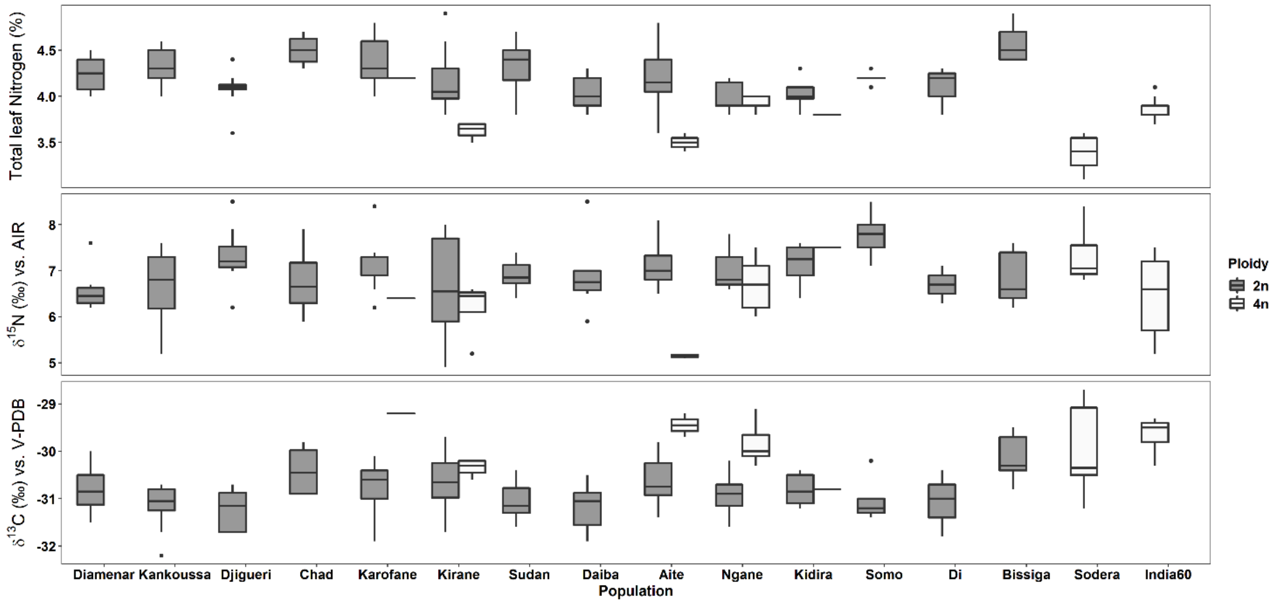

Fig. 4 Box plot of variation in carbon and nitrogen isotope ratios and total leaf nitrogen between populations and ploidy levels. Lines represent provenances with only one tree at the ploidy level. Box plots represent median, first-third quartile, minimum and maximum values. Gray and white boxes represent diploid and tetraploid trees, respectively. Populations were ordered by annual mean precipitation. Please note that the number of trees per population and ploidy level differ (cf. Table 1) and that differences among populations were not significant for δ^{15}N (both ploidy levels) and for δ^{13}C (tetraploids).

Among diploid populations, δ^{13}C and total leaf N varied significantly, while δ^{15}N did not show significant differences (Table 3). The populations Chad and Bissiga (Burkina Faso) had the highest mean values of δ^{13}C and total leaf N, and the local (relative to the country of the trial site) populations from Senegal (Daiba, Ngane and Kidira) and the Djigueri population from Mauritania had the lowest values of δ^{13}C and total leaf N (Fig. 3, Fig. S1 shows post hoc tests). Only the total leaf N differed among tetraploid populations, and no significant differences were found in δ^{13}C and δ^{15}N (Table 3). The local population Ngane displayed the highest total leaf N, and lowest values were found in populations Aite (Mali) and Sodera (Ethiopia) (Fig. 4, Fig. S2 shows post hoc tests). Effects of blocks were not significant for any of the traits among both diploid and tetraploid populations (Table S4).

3.4. Multivariate analysis

The leaf traits were highly correlated. The first two components in the Principal Component Analysis (PCA) explained a high amount of the variation in leaf traits, but without exposing clear difference between diploid and tetraploid populations (Fig. 5).

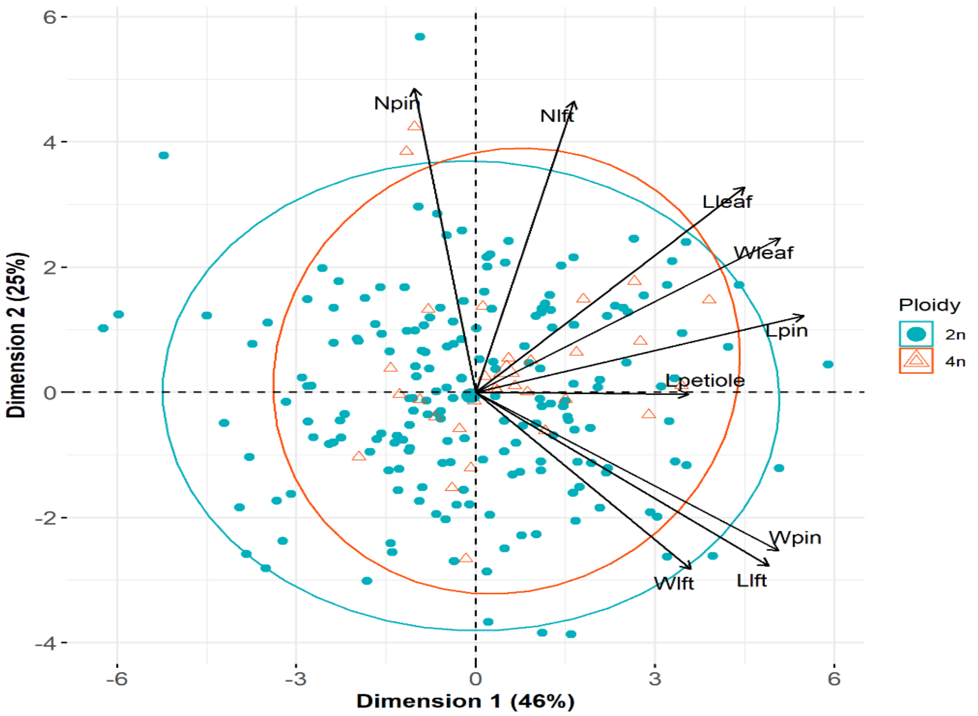

Fig. 5 Plot of the principal components one and two among ploidy level. The directions of the arrows show the relative loadings of the leaf traits. Npin = number of pinnae per leaf; Nlft = number of leaflets per leaf; Lpetiole = petiole length; Lleaf = leaf length; Wleaf = leaf width; Lpin = pinnae length; Wpin = pinnae width; Llft = leaflet length; Wlft = leaflet width.

In the canonical variate analysis, the first four canonical variates were significant, and the plot of the two first canonical variates showed that diploids and tetraploids can to some extend be differentiated by their leaf morphology (Fig 6). This was confirmed by the Wilks' Lambda statistic (p = 0.0044). However, the significance was not much different from the univariate tests of difference in petiole and leaf length (cf. Table 3). The Euclidean distances between diploid and tetraploid means from the same populations were not significantly different from distances between random pairs of diploid and tetraploids (p = 0.9239). Hence, there is no indication that diploids and tetraploids from the same populations are more alike than diploids and tetraploids from different populations (see also Fig. 6).

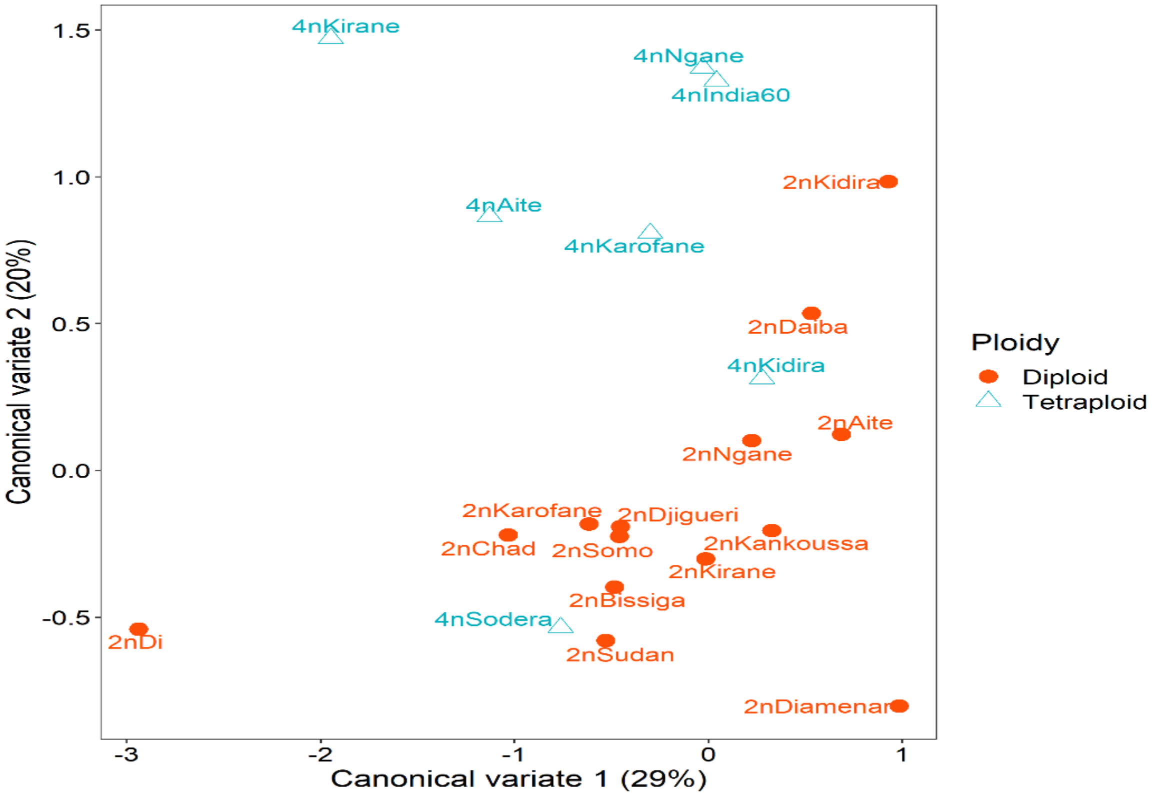

Fig. 6 Canonical scores of the diploid and tetraploid populations, plotted against the two first canonical variates

3.5. Geographic and climatic patterns

The correlation analyses (Table 4) revealed that many of the leaf morphological characters were significantly correlated, especially for diploid populations. The number of significant correlations were lower among tetraploids compared to diploids, but this can be due to the lower power of the analyses as there were fewer tetraploid individuals.

In tetraploid populations there was no significant correlation of the morphological traits with climatic and geographical parameters of origin. In diploid populations, however, number of leaflets and leaf length correlated significantly and negatively with latitude of origins, meaning that populations from southern sites tended to have longer leaves and more leaflets compared with northern populations. In addition, leaflet length correlated negatively with altitude, populations from high altitude having smaller leaflets compared to populations from low altitude (Fig. 7). Leaf length correlated significantly and positively with annual mean precipitation and the aridity index, i.e. leaf length increased from drier to wetter sites of origin (Fig. 7).

Table 4 Pairwise correlations given by Pearson's correlation coefficient at the population means between leaf measured traits, geographical parameters and isotope ratios (δ^{13}C, δ^{15}N) composition in diploids (upper right triangle) and tetraploids (lower left triangle)

	Petiole length	No. of pinnea	No. of leaflets	Leaf length	Leaf width	Leaflet width	Leaflet length	Pinnea length	Pinnea width	Annual Prec_mean	Annual Temp_mean	Aridity Index	Longitude	Latitude	Altitude	δ^{13}C	δ^{15}N	Total N
Petiole length	-	-0.22	0.03	0.51	0.52	0.28	0.26	0.37	0.28	0.42	-0.36	0.44	0.23	-0.5	0.24	0.07	0.61*	-0.07
No. pinnea	-0.6	-	0.72**	0.36	-0.25	-0.73**	-0.78***	-0.28	-0.79***	0.48	-0.18	0.49	0.16	-0.51	0.28	0.16	-0.1	0.16
No. leaflets	0.31	0.33	-	0.33	-0.04	-0.67**	-0.64*	-0.11	-0.63*	0.29	-0.08	0.29	0.49	-0.63*	0.48	0.22	-0.2	0.31
Leaf length	0.54	0.16	0.65	-	0.73**	0.2	0.11	0.69**	0.15	0.61**	-0.27	0.62*	0.26	-0.74**	0.23	0.62*	0.32	0.31
Leaf width	0.58	-0.03	0.86*	0.68	-	0.61*	0.64*	0.93***	0.71**	0.14	0.1	0.13	0.08	-0.3	-0.06	0.55*	0.32	0.27
Leaflet width	0.72	-0.95***	-0.24	-0.12	0.16	-	0.83***	0.71**	0.85***	-0.19	-0.08	-0.19	-0.17	0.22	-0.27	0.27	0.15	0.1
Leaflet length	0.58	-0.64	0.11	0.48	0.47	0.55	-	0.71**	0.98***	-0.15	0.29	-0.17	-0.37	0.37	-0.56*	0.15	0.21	-0.16
Pinnea length	0.59	-0.32	0.71	0.63	0.87*	0.31	0.74	-	0.74**	0.12	0.04	0.11	-0.03	-0.2	-0.22	0.63*	0.1	0.29
Pinnea width	0.67	-0.69	0.13	0.47	0.51	0.64	0.99***	0.76*	-	-0.19	0.31	-0.21	-0.3	0.32	-0.47	0.19	0.23	-0.09
Annual Prec_mean	-0.17	-0.47	-0.35	-0.36	0	0.37	0.45	0.18	0.42	-	-0.23	0.99***	-0.1	-0.61*	0.09	0.24	0.33	-0.11
AnnualTemp_mean	0.17	0.34	0.35	0.69	0.08	-0.42	0.07	0.17	0.04	-0.71	-	-0.29	-0.38	0.47	-0.43	0.04	-0.1	-0.09
Aridity Index	-0.19	-0.44	-0.35	-0.47	-0.01	0.39	0.32	0.1	0.31	0.98***	-0.84*	-	-0.07	-0.63*	0.12	0.23	0.34	-0.1
Longitude	-0.43	-0.16	-0.56	-0.5	-0.35	0.11	-0.03	-0.3	-0.07	0.79*	-0.67	0.81*	-	-0.69**	0.87***	0.17	-0	0.57*
Latitude	-0.35	0.11	-0.35	0.19	-0.31	-0.29	0.24	-0.11	0.14	0.35	0.23	0.2	0.53	-	-0.7**	-0.39	-0.2	-0.44
Altitude	-0.09	-0.16	-0.12	-0.63	0.04	0.32	-0.34	-0.2	-0.27	0.39	-0.91**	0.56	0.44	-0.51	-	0.17	0.14	0.58*
δ^{13}C	-0.79*	0.36	-0.61	-0.69	-0.91**	-0.46	-0.74	-0.88**	-0.79*	-0.13	-0.05	-0.09	0.27	0.19	0.05	-	-0.2	0.66**
δ^{15}N	0.7	-0.9**	-0.15	-0.06	0.3	0.97***	0.61	0.39	0.69	0.49	-0.52	0.52	0.19	-0.26	0.41	-0.58	-	-0.23
Total N	0.2	-0.38	-0.64	-0.02	-0.61	0.27	0.1	-0.37	0.1	-0.22	0.38	-0.3	-0.04	0.35	-0.48	0.31	0.09	-

Note: the values for tetraploids are based on only 7 populations

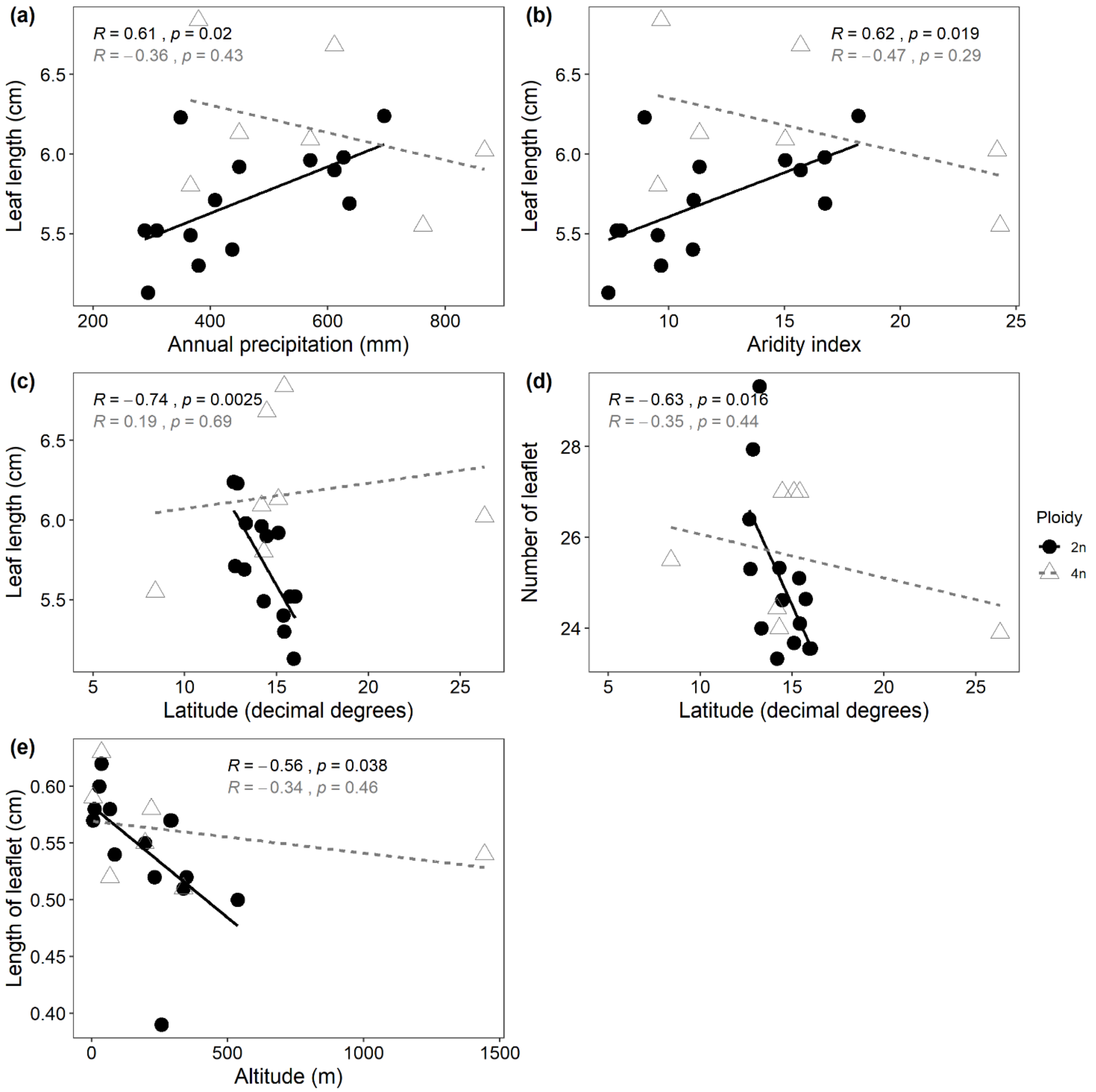

Fig. 7 Pairwise plots of, leaf length against annual precipitation (a) and aridity index (b), and latitude (c), number of leaflet against latitude (c), and length of leaflet against altitude (e) in diploid and tetraploid populations. Symbols represent population means.

The total leaf N content was positively correlated with the longitude and altitude in diploid populations. No significant correlations were observed for total N in tetraploids. In diploid populations, δ^{13}C was positively correlated with the length and width of the leaf and the pinnae length, and δ^{15}N increased with the length of the petiole. Conversely, in tetraploid populations, δ^{13}C was negatively correlated with petiole length, leaf width, and pinnae length and width. δ^{15}N was negatively correlated with the number of pinnae and positively correlated with the leaflet width. Hence, tendencies of δ^{13}C and δ^{15}N between populations seem to be different in diploids and tetraploids (Fig. 8). However, when comparing the patterns of diploid and tetraploid populations, it should be recalled that nine populations did not have tetraploids and two populations did not have diploids. This means that the analyses of environmental associations for diploid and tetraploid trees do not sample the same environments. The purely tetraploid Sodera (Ethiopia) and India populations thus represent origins from areas with quite lower aridity index, and Sodera also from high altitude, compared to any of the diploid populations (Table 1).

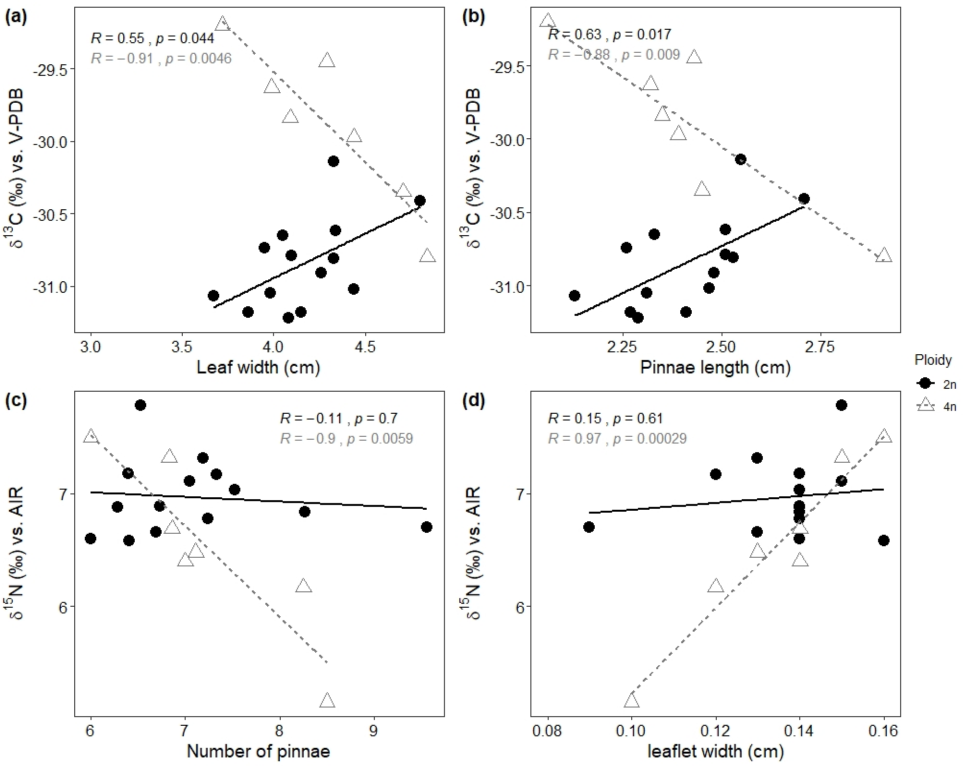

Fig. 8 Pairwise plots of the stable carbon isotope composition against leaf width (a) and pinnae length (b) and nitrogen isotope ratio against number of pinnae (d) and leaflet width (d) in diploid and tetraploid populations. Symbols represent population means.

4. Discussion

4.1. Geographic distribution of polyploidy among populations

The present study documents frequent coexistence of different ploidy levels in populations of *A. senegal*, and thereby support the findings of Assoumane et al. (2012), Odee et al (2015) and Diallo et al. (2016). Furthermore, using a larger sample size than previously applied, we confirm that some populations appear to be predominantly di- or tetraploid, while others have mixed ploidies. Our finding of pure tetraploid populations in the East African populations (Sodera, Ethiopia) supports the observations by Odee et al (2015) of no mixed ploidy populations detected in the southern range of the species. It is interesting that we observe a high frequency of tetraploids in three populations from wet areas (Ngane, Sodera and India60). We do not know the ploidy level of all trees at the time of establishment of the common garden trial (our estimate is only based on surviving trees), but an *in situ* study of the Ngane population (also included in the present study) has reviously reported a high frequency of polyploids (Diallo et al. 2015) which supports our findings.

4.2. Phenotypic variation in leaf characters among ploidy levels

The small morphological differences between di- and tetraploid trees can explain why the presence of different ploidy levels in *Acacia senegal* were only recently recognised.

The leaf morphology of the rare triploid trees (we identified six trees) resembled the botanical description of *A. laeta* leaf morphology by Giffard (1966), with pinnea having at least three to five widely elliptical leaflets. One hyphothesis is that the triploid individuals observed in this study has a hybrid origin between *A. senegal* var. *senegal* and *A. mellifera* subsp. *mellifera*, a hybrid first recognized by Kan (1951) (here after Fagg and Allison 2004). This hybrid has by other authors been referred to as *A. laeta* (El Amin 1976 in Fagg and Allison 2004; Chevallier et al. 1994; Mensous et al. 2017), which is considered to be of allopolyploid origin (Chevallier and Borgel 1998; Assoumane et al. 2012; Odee et al. 2012, 2015).

However, it is also possible that the identified triploids in our study indeed are a natural cytotype of *A. senegal*. Odee et al. (2015) found triploid individuals in the Karofane population, suggesting that the triploids could have arisen either from a diploid × tetraploid cross, or from the combination of reduced and unreduced gametes of a diploid. We found additional triploid individuals in the Karofane and Somo populations, and both populations also had diploid and polyploid individuals. Studies based on genetic markers in combination with the sequencing of the internal transcribed spacer (ITS) region can help to clarify if the triploid individuals have a hybrid origin (Turchetto et al. 2015; Du et al. 2010). It will also be highly relevant to compare the leaf morphology from previously reported triploid *Acacia senegal* trees with leaf morphology of the triploid trees reported in the present study.

4.3. Adaptive potential of *A. senegal* to dry environments

Leaf morphology varied according to precipitation and latitudinal gradients at the site of origin with smaller leaves and fewer leaflets per pinnae on dryer sites in diploid populations. This genetic differentiation in leaf characters could reflect a local adaptation of *A. senegal* to dry conditions. It may represent a general feature of Sahelian tree species, because similar observations has been made in *Parkia biglobosa* (Teklehaimanot et al. 1998) and *Adansonia digitata* (Cuni Sanchez et al. 2010; Korbo et al. 2011), which showed differences in the number of leaflets per leaf between Northern and Southern origins.

Natural selection can shape differences in leaf morphology (Arntz and Delph 2001) and plants in dry areas tend to have smaller leaves compared to plants from humid areas (McDonald et al. 2003). Drought-resistant plants often have different leaf anatomy and morphology, a higher gas exchange rate and greater osmotic potentials compared to drought-sensitive plants in dry conditions (Abrams et al. 1990). The smaller leaves of diploid *A. senegal* trees from sites with low precipitation can therefore reflect adaptation to stressful Sahelian conditions, for example by reducing excessive heating of leaves. A study of West African *P. biglobosa* found that Northern populations (from more dry condition) showed more xerophytic characteristics with smaller height, smaller leaflets and greater number of leaflets per pinnae, lower specific leaflet mass and smaller palisade tissue (Teklehaimanot et al. 1998). Studies of *A. senegal* that include more detailed anatomical observation combined with ecophysiological tests will be important in order to better understand the role of leaf morphology as a means to adapt to dry sites.

It is intriguing that the patterns of environmental correlations observed for diploid *A. senegal* were not observed for tetraploids. This may be result of high rate of asexual reproduction (apomixes) in tetraploids (Assoumane et al 2012; Diallo et al. 2015) that at least in theory will reduce the adaptive potential in tetraploids. However, as mentioned above, care must be taken when comparing the pattern of diploid and tetraploid trees, because the populations partly sample different environments.

The difference in leaf morphology between diploid and tetraploid *A. senegal* in the present study corresponds to the findings of Diallo et al. (2016) who also found that diploid *A. senegal* differed from polyploids by smaller leaflets, but also higher density and smaller size of stomata.

The total leaf N content was slightly lower in tetraploids compared to diploids, and tetraploid populations displayed the lowest leaf δ^{15}N. While this could indicate that diploids and tetraploids differ in sourcing of nitrogen, the small differences in mean values between ploidy levels call for additional studies. The δ^{15}N values of leaves reflect ^{15}N/^{14}N preserved during N absorption, assimilation and translocation of the N source in the soil (Evans 2001). Leguminous species obtain their N from the soil and through symbiotic fixation, and the two N sources usually differ in ^{15}N abundance (Raddad et al. 2005). Raddad et al. (2005) found that the ability of *A. senegal* to fix N under natural conditions makes it less dependent on soil N, compared to a non-N_2-fixing plant *Balanites aegyptiaca* in Sudan. In a study in the common garden trial in Dahra/Senegal (also used for the present investigation), Gray et al. (2013) came to the opposite conclusion. The δ^{15}N value from *A. senegal* (6.77 ± 0.50‰) was higher than the non-N_2-fixing reference tree *Balanites aegyptiaca*, suggesting no biological N fixation, perhaps as a consequence of high temperatures and drought stress. Our mean δ^{15}N values for diploids (6.9 ± 0.68) and tetraploids (6.6 ± 0.83) are close to the values found by Gray et al. (2013), considering that Gray et al. (2013) did not take the ploidy level into account. The indication that diploid and tetraploid trees source their N differently clearly calls for experiments on nodulation and root growth of diploid and tetraploid trees.

The success of legumes as drought-deciduous shrubs in dry environments depends on the ability to photosynthesize rapidly during favorable periods, while avoiding excessive water loss, for example by shedding leaves during unfavorable periods (Dupuy and Dreyfus 1992; Mckey 1994). C isotopic composition differed substantially among trees in our study, to a large extent as a consequence of ploidy level. Sarr et al. (2021) suggested that others factors may impact *A. senegal* WUE, since only 15% of the variation in isotopic composition was related to variation in ploidy level. Even when combined, the study of Sarr et al. (2021) and the study presented here represent a limited sample of tetraploid individuals, and further investigations will be needed to clarify the implication of poplyploidy in adaptive traits of *A. senegal*.

In quaking aspen (*Populus tremuloides* Michx.), Greer et al. (2018) found that ploidy level strongly influences physiological traits and function as a response to environmental conditions. Despite triploid aspen displaying a higher water use efficiency compared to diploids, they also had greater potential water loss because of higher stomatal conductance and lower stomatal sensitivity to increasing vapor pressure deficit. While diploid and tetraploid trees in our study had leaves that looked almost similar, the iWUE was significantly higher in tetraploid individuals. Although we did not observe a clear correspondance between leaf morphology and carbon isotope composition, there appeared to be a relationship between δ^{13}C and geographic origin in diploid populations. Leaf size affects the thickness of the boundary layer and hence diffusion of gases in and out of leaves, affecting the iWUE (Schuepp 1993). Surprisingly, the different trends of δ^{13}C in diploid and tetraploid populations in relation to leaf size (Fig. 6a,b) does not suggest a coherent response of iWUE to leaf size. Differences in stomatal and mesophyll conductances to CO_2 might cause changes in δ^{13}C and iWUE (Seibt et al. 2008), and ploidy level is affecting mesophyll structure and architecture (Khramtsova et al. 2003). It has previously been shown that tetraploids in *A. senegal* have fewer and larger stomata compared to diploids, which should lead to higher iWUE (Diallo et al. 2016), but it is also possible that tetraploids have lower mesophyll conductances than diploids, leading to the same result. Across a range of *Acacia* species, Konaté et al. (2016) observed that δ^{13}C and iWUE were correlated to stomatal conductance under saturating light condidions, suggesting an important role for stomata. However, the measured differences in δ^{13}C between ploidy levels can also be influenced by the differences in petiole length, because petioles were included in the sample preparation, and we cannot exclude that a higher content of lignin in petioles compared to pinnae could influence the C isotopic composition.

It is important to note that the higher iWUE does not necessarily lead to slower growth, and tetraploids have proven to grow faster than diploids under different conditions (Diallo et al. 2016). Differences in leaf N content and WUE could also be caused by root architecture, as deep roots may provide access to other sources of water, allowing plants to keep more stomata open longer and thus capture CO_2 at a higher rate. As a follow-up research we therefore suggest studies of leaf conductance, root/shoot ratio and root architecture of diploid and tetraploid *A. senegal.* Likewise, studies of leaf phenology can give insight in the leaf lifespan and thus strategies of the two ploidy levels. Finally, sampling of leaves for WUE measurements in the rainy and dry season may reveal different patterns among populations and level of ploidy. In this context, Gray et al. (2013) found that population WUE estimates were different depending on the sampling time.

The fact that the relationships between leaf morphology, total leaf N, δ^{13}C, and δ^{15}N and climatic and geographic parameters are different in diploid and tetraploid populations suggests that the two ploidy levels may have developed different adaptation strategies to the harsh Sahelian growing conditions. This is supported by the multivariate analysis that showed that pairs of diploids and tetraploids from the same populations are not morphologically alike. Earlier studies have suggested that gene flow between the two cytotypes is likely to be very limited (Assoumane et al. 2012; Diallo et al. 2015). Cytotype reproductive isolation and adaptation to different niches is frequently implied in speciation (Ramsey and Schemske 1998), and *A. senegal* may be a case where selection is working in different directions in the two cytotypes (cf. Arntz and Delph 2001). Whether this is happing in *A. senegal* will require additional studies on adaptive traits in combination with genomic studies.

5. Conclusion

Our study showed that leaf morphology in *A. senegal* varied among ploidy levels and among diploid populations. We interpret this as a result of local adaptation, but also as an indication of differences between diploid and tetraploid individuals in their strategies to overcome drought. Leaf length and number of leaflets per pinnae varied according to precipitation and latitudinal gradient at the site of origin in diploid populations, while a similar covariation was not proven significant among tetraploid trees. Compared to diploids, the tetraploid trees had higher WUE and lower leaf N content, which are characteristics for adaptation to dry conditions. The differences may not only be related to macro leaf morphology, as other factors such as micromorphological features and availability of N sources could also be involved. Given the importance of the tree species and the magnitude of on-going climatic changes, we recommend more studies to adress adaptation of *A. senegal* to dry growing conditions.

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Supplementary material

Table S1 Test of autoregressive correlations for the different traits using a linear mixed model with an effect of provenance, block and provenance by block interaction and using data from diploid trees only. Model 1 below has an added autoregressive correlation matrix and model 2 no autoregressive correlation matrix. The significance of the added autoregressive correlation matrix is tested a χ^2- test with two degrees of freedom.

Trait	First-order autoregression correlation coefficients		Loglike-lihood model 1	Loglike-lihood model 2	2 x diffe-rence model 1 - model 2	P-value
Number of pinnea	-0.17	0.15	-139.2	-140.6	2.8	0.2424
Number of leaflets	-0.06	-0.24	-258.5	-259.5	2.0	0.3639
Petiole length	0.03	0.06	95.0	94.9	0.2	0.9274
Leaf length	-0.12	0.15	-47.2	-47.8	1.3	0.5205
Leaf width	0.11	0.02	14.4	14.2	0.6	0.7568
Pinnea length	0.06	-0.07	106.9	106.7	0.3	0.8607
Pinnea width	0.05	0.13	259.1	258.6	0.9	0.6238
Leaflet length	0.23	0.13	384.2	382.8	2.8	0.2528
leaflet width	0.13	-0.23	223.3	222.3	2.2	0.3403
Total leaf N	0.48	-0.20	68.2	67.1	2.2	0.3307
$\delta^{15}N$	0.16	-0.74	-17.0	-19.4	4.7	0.0942
$\delta^{13}C$	-0.33	-0.67	5.3	2.6	5.5	0.0639

Table S2 Studentized residuals of all morphological traits for the two outliers (Karofane1 block 4 and Djigueri 5 block 1) from the model 4 (diploid populations)

Trait	TreeID	Studentized residual
Number leaflets	Karofane41	-5.2
	Djigueri15	-4.9
Number pinnea	Karofane41	-2.76794
	Djigueri15	-0.10678
Petiole length	Karofane41	8.1
	Djigueri15	4.9
Leaf length	Karofane41	8.2
	Djigueri15	7.1
Leaf width	Karofane41	10.8
	Djigueri15	6.1
Pinnea length	Karofane41	11.3
	Djigueri15	5.2
Pinnea width	Karofane41	11.2
	Djigueri15	7.8
Leaflet length	Karofane41	11.5
	Djigueri15	7.5
Leaflet width	Karofane41	5.3
	Djigueri15	3.7

Table S3 Variation in leaf morphology, total leaf N, δ^{13}C and δ^{15}N of *Acacia senegal* from mixed populations (Aite, Karofane, Kidira, Kirane and Ngane) (model 3 including populations with a mix of diploid and tetraploid trees only).

Trait	Source	Df	SS	MS	F value	P value
Petiole length	Population	4	0.2746	0.0686	0.77	0.57052
	Ploidy	1	0.8320	0.8320	9.35	**0.00324****
	Block	3	0.8812	0.2937	3.28	0.063156
Number of pinnea	Population	15	8.7714	2.1928	1.14	0.3889
	Ploidy	1	2.1054	2.1054	1.10	0.2984
	Block	3	2.1948	0.7316	0.38	0.7687
Number of leaflet	Population	15	16.0572	4.0143	0.78	0.56161
	Ploidy	1	19.3027	19.3027	3.74	0.05763
	Block	3	8.9705	2.9902	0.58	0.64024
Leaf length	Population	15	0.7888	0.1972	0.47	0.75477
	Ploidy	1	3.0578	3.0578	7.34	**0.00868****
	Block	3	0.6291	0.2097	0.50	0.68804
Leaf width	Population	15	0.4183	0.1046	0.49	0.7446
	Ploidy	1	0.2692	0.0897	0.42	0.7433
	Block	3	0.0815	0.0815	0.38	0.5394
Leaflet length	Population	15	0.0516	0.0129	4.94	**0.01588***
	Ploidy	1	0.0003	0.0003	0.12	0.73079
	Block	3	0.0060	0.0020	0.76	0.53783
Leaflet width	Population	15	0.0023	0.0006	1.78	0.208
	Ploidy	1	0.0008	0.0003	0.80	0.5191
	Block	3	0.0006	0.0006	1.90	0.1729
Pinnea length	Population	15	0.2581	0.0645	0.98	0.4568
	Ploidy	1	0.0084	0.0084	0.13	0.7213
	Block	3	0.0768	0.0256	0.39	0.7633
Pinnea width	Population	15	0.2052	0.0513	3.84	**0.03875***
	Ploidy	1	0.0006	0.0006	0.05	0.82926
	Block	3	0.0275	0.0092	0.68	0.58029
δ^{13}C	Population	15	0.5137	0.1284	0.48	0.7495
	Ploidy	1	6.0529	6.0529	22.77	< **0.0001*****
	Block	3	0.4916	0.1639	0.61	0.6202
δ^{15}N	Population	15	2.2897	0.5724	1.18	0.37783
	Ploidy	1	2.8923	2.8923	5.99	**0.01832***
	Block	3	1.6733	0.5578	1.15	0.37307
Total N	Population	15	0.7013	0.1753	2.70	0.0935878
	Ploidy	1	0.8306	0.8306	12.85	**0.0008*****
	Block	3	0.6690	0.2230	3.44	0.0566014

Table S4 Variation in leaf morphology, total leaf N, δ^{13}C and δ^{15}N of *Acacia senegal* among diploid and tetraploid populations (model 4).

Trait	Source	**Diploid (2n)**					**Tetraploid (4n)**				
		Df	SS	MS	F value	P value	Df	SS	MS	F value	P value
Petiole length	Population	13	1.1394	0.0876	0.88	0.5838	6	0.2569	0.0428	0.83	0.5943
	Block	3	0.2765	0.0922	0.92	0.4418	3	0.3372	0.1124	2.19	0.2282
Number of pinnea	Population	13	47.3320	3.6409	2.33	**0.02603***	6	10.1390	1.6899	0.81	0.6031
	Block	3	1.8410	0.6138	0.39	0.7578	3	4.2230	1.4077	0.68	0.6073
Number of leaflet	Population	13	219.8530	16.9117	2.82	**0.00871****	6	39.2720	6.5453	1.96	0.2441
	Block	3	10.1040	3.3678	0.56	0.6435	3	1.4090	0.4695	0.14	0.9301
Leaf length	Population	13	10.7417	0.8263	1.61	0.1336	6	5.5653	0.9275	2.37	0.1873
	Block	3	1.1877	0.3959	0.77	0.518	3	2.0658	0.6886	1.77	0.2887
Leaf width	Population	13	5.4601	0.4200	1.71	0.1064	6	1.8245	0.3041	1.83	0.2678
	Block	3	0.9265	0.3088	1.26	0.3061	3	0.1099	0.0366	0.22	0.8771
Leaflet length	Population	13	0.1798	0.0138	4.14	**0.00052*****	6	0.0145	0.0024	0.97	0.5278
	Block	3	0.0098	0.0033	0.98	0.413845	3	0.0098	0.0033	1.31	0.3837
Leaflet width	Population	13	0.0243	0.0019	4.00	**0.00094*****	6	0.0029	0.0005	3.54	0.0991
	Block	3	0.0032	0.0011	2.31	0.1034762	3	0.0017	0.0006	4.08	0.1008
Pinnea length	Population	13	1.1508	0.0885	1.14	0.3655	6	0.3597	0.0600	1.02	0.5028
	Block	3	0.1116	0.0372	0.48	0.6996	3	0.0985	0.0328	0.56	0.6665
Pinnea width	Population	13	0.7412	0.0570	3.69	**0.00157****	6	0.0444	0.0074	0.74	0.6424
	Block	3	0.0572	0.0191	1.24	0.316662	3	0.0206	0.0069	0.69	0.6021
δ^{13}C	Population	13	8.0607	0.6201	2.42	0.02392*	6	1.1619	0.1937	0.77	0.6197
	Block	3	0.6000	0.2000	0.78	0.51285	3	0.1384	0.0461	0.18	0.9041
δ^{15}N	Population	13	7.8918	0.6071	1.47	0.1885	6	5.1190	0.8532	3.17	0.0863
	Block	3	2.3718	0.7906	1.92	0.1465	3	0.6683	0.2228	0.83	0.5291
Total N	Population	13	2.0626	0.1587	3.21	**0.00380****	6	1.0790	0.1798	10.42	**0.01223***
	Block	3	0.3259	0.1086	2.20	0.106879	3	0.0799	0.0266	1.55	0.3295

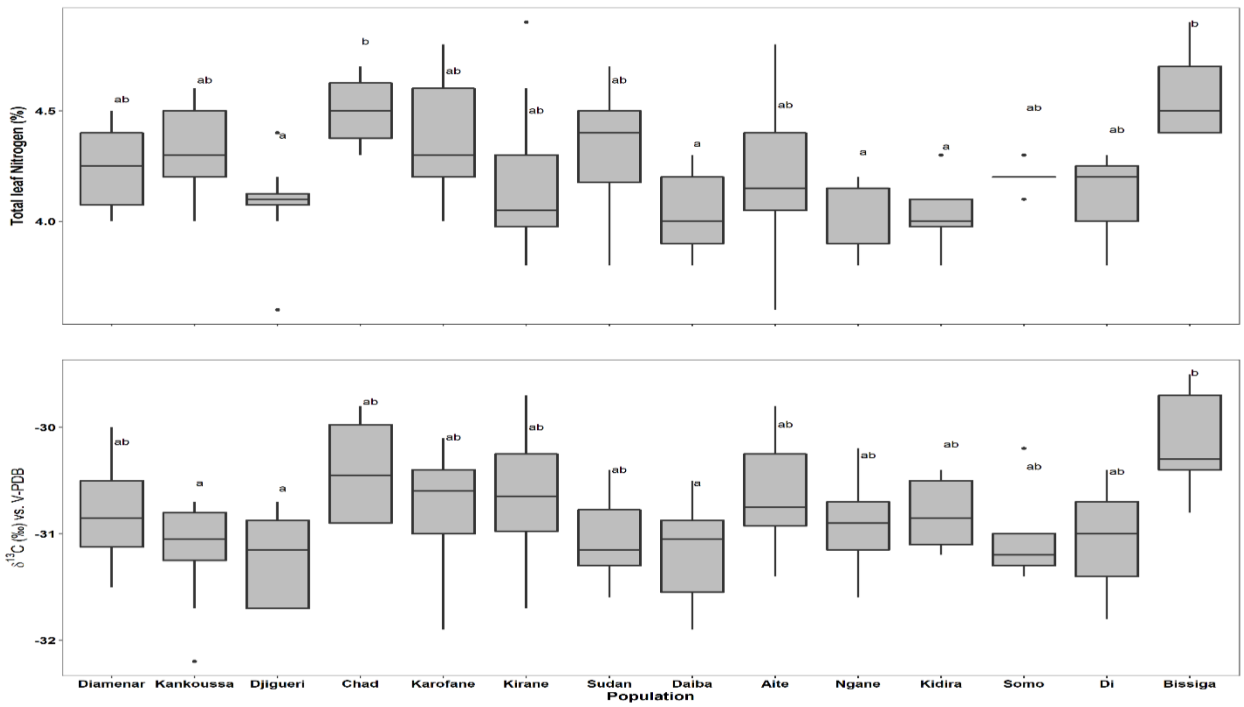

Fig. S1 Box plot of variation in δ¹³C and total leaf nitrogen among diploid populations. Box plots represent median, first-third quartile, minimum and maximum values. Populations were ordered by annual mean precipitation.

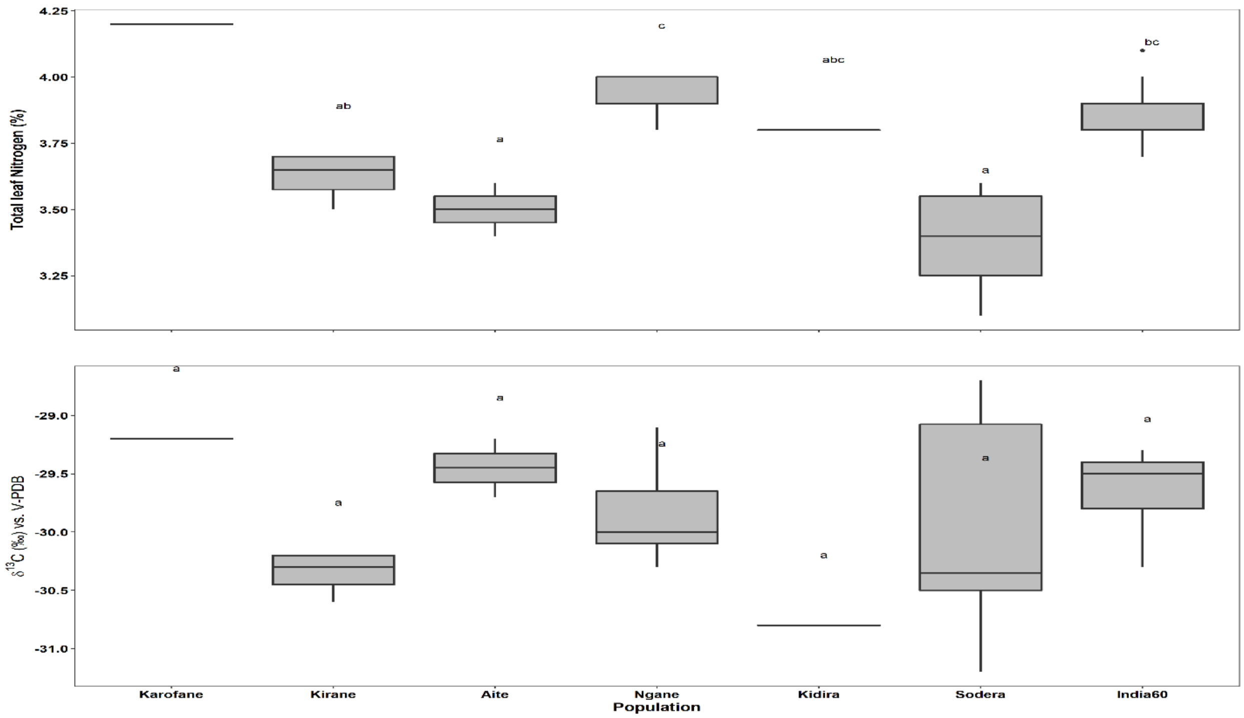

Fig. S2 Box plot of variation in δ¹³C and total leaf nitrogen among tetraploid populations. Box plots represent median, first-third quartile, minimum and maximum values. Populations were ordered by annual mean precipitation.

Manuscript II. Survival and growth of *Acacia senegal* (L.) Wild. (*Senegalia senegal* (L.) Britton) provenances depend on the rainfall at the site of origin.

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***Key message*:** Variation among provenances of *Acacia senegal* (*Senegalia senegal* (L.) Britton) in their survival, and growth in height and diameter at two sites in Senegal could be partly explained by the climate at their site of origin and also revealed genotype-by-environment interactions. Breeding programs and forest restoration projects should carefully select germplasm for optimal performance of this socioeconomically important tree species.

Abstract

Context: Several studies have shown important differences in adaptive properties among provenances (origins) of African savanna tree species, but there is a lack of studies on how these differences are expressed in different environments and age, and to what extent they reflect local adaptation.

Aims: This study investigates the survival and growth of trees from 15 African *Acacia senegal* (*Senegalia senegal* (L.) Britton) provenances tested in two environments and relates the differences among provenances to the climate at their site of origin and tree age.

Methods: The study is based on 14 years growth in a common garden trial at two sites (Bambey and Dahra, Senegal) that differ in water availability, followed up by later assessment after 23 years at one of the sites.

Results: The variation among provenances in survival, height and diameter was significant and differences could be partly explained by the climate at their site of origin. In general, provenances from dry sites survived better at both sites. However, we observed genotype-by-environmental interactions where provenances from dry sites on average performed relatively poorer in height and diameter at the wettest site (Bambey) compared to the drier site (Dahra), while the opposite was the case for provenances from wetter sites.

Conclusion: The results support that divergent selection creates and maintains local adaptation of *Acacia senegal* provenances in relation to growth (height and diameter) and survival in areas with different water availability. This has important implications for choice of appropriate planting material for tree planting and for conservation of genetic variation among natural populations, but also for prediction of the effects of climate change.

Keys words: Adaptation, Africa, Climate, Genotype-by-environment interaction, *Senegalia senegal*.

1. Introduction

Africa is reported to be a continent where half of the forest species are threatened (FAO 2014), and the Sudano-Sahelian region is one of the most vulnerable zones characterized by severe changes due to climate variability and human activities (Mertz et al. 2011; Bégué et al. 2011). For centuries, the Sudano-Sahelian region has experienced high rainfall variability (Tschakert 2007; Mertz et al. 2009), and adaptive strategies to respond to harsh natural conditions is important. For plants, drought stress is a factor that strongly limits survival and productivity (Khan et al. 2010), particularly in Senegal where drought is believed to have reduced forest species richness and tree density markedly during the last half of the 20th century (Gonzalez 2001). Still, recent findings and climate projections suggest a trend of increasing vegetation greenness in arid and semi-arid Sahel (Olsson et al. 2005; Kaptué et al. 2015; Pausata et al. 2020) with 30% of woody tree cover in the sub-humid zone (Brandt et al. 2018). Although the recent observed regreening trends in the Sahel region may reflect both changes in rainfall and land management, it pinpoints the uncertain and variable nature of growth conditions in the region. Large scale planting program such as the Great Green Wall (GGW) aims to contribute to the reforestation efforts and reduction of land degradation in Sahelian regions reinforcing the greening tendency through planting of multipurpose tree species (O'Connor and Ford, 2014). In Senegal the implementation of the GGW has focused on planting of 26 ligneous plant species including the well-known gum arabic tree, *A. senegal*, but so far only 119.000 ha, i.e. 15% of the targeted degraded lands (817.500 ha) have been restored (PAGGW, 2020).

Improved insight in variation among provenances of Sahelian tree species is important in order to support conservation of valuable gene pools with special adaptive features, but also to guide selection and development of suitable seed sources (provenances) for future afforestation and reforestation of wooded landscapes. Natural variation among trees in adaptive traits is important to ensure that a species can cover heterogeneous environments, and is essential for a species' ability to respond to new environmental conditions through selection of superior phenotypes.

Phenotypic variation is the combined effect of genetic and environmental heterogeneity and their interaction (G×E interaction). When studied under controlled environmental conditions, the variation among phenotypes can reveal underlying genetic variation (Dangasuk et al. 1997; Westoby et al. 2002; Stöcklin et al. 2009). By comparing the phenotypic performance of different populations within and among different environments (test sites), it is further possible to quantify and qualify the genotype-by-environment interactions (G×E) and thereby infer on past and ongoing adaptation of species to their physical environment (Westoby et al. 2002; Raddad 2007; Stöcklin et al. 2009).

By the use of hundreds of provenance trials (also known as common garden trials), it has been possible to compare phenotypic performance of different origins at multiple sites in temperate and boreal forests during the past century, and results have revealed eco-geographic patterns of genetic variation for many species (e.g. Langlet 1971; Morgenstern 1996; Risk et al. 2021). Provenance trials have also been widely applied to test to what extent tree species will be able to adapt to the ongoing climate changes (e.g. Matyas 1994; Rweyongeza et al. 20011 ; Lobo et al. 2018), and to improve early selection (Chen et al. 2004; Dong et al. 2019). Most of the studied tree species have large geographic distribution areas that encompass substantial ecological variation, and the provenance trials have therefore in general not identified a single outstanding provenance – rather provenance tests have revealed that different provenances are suitable in different planting zones due to G×E interactions (e.g., Matheson and Raymond 1986; Wu and Matheson 2005). Understanding these patterns of adaptive genetic variation is essential for the development of site specific provenance recommendations, delimitation of breeding zones, seed transfer guidelines for afforestation and reforestation, as well as to guide conservation programs (e.g. Burdon 1977; Matheson and Raymond 1986; Rehfeldt et al. 1999; Ying and Yanchuk 2006; Malaval et al. 2010).

Unfortunately, much less is known about the geographic and ecological patterns of variation in Sudano-Sahelian tree species although the need for wise deployment and conservation of the species is immense. Several studies of tree species from the region have shown provenance variation in growth and survival, *i.e. Faidherbia albida* (Delile) A. Chev. (Billand and De Framond 1993), *Prosopis africana* (Guill. & Perr.) Taub. (Weber et al. 2008; Sotelo Montes and Weber 2009), *Balanites aegyptiaca* (L.) Delile (Weber and Sotelo Montes 2010), *Acacia senegal* (Larwanou et al. 2010; Raebild et al. 2003a,b,c), *Parkia biglobosa* (Jacq.) Benth (Ouedraogo et al. 2012), *Vachellia nilotica* (L.) Willd. ex Delile (Larwanou et al. 2014). Observed phenotypic variation can often be associated with clinal patterns of environmental variation. Weber et al. (2008) and Sotelo Montes and Weber (2009) found clinal variation in growth and survival in *P. africana* in Niger. These studies suggested that, under dry conditions, provenances from drier areas performed better in growth and survival compared to provenances from the wetter parts of Niger and Burkina Faso. The same trends were found in 13 years old provenances of *B. aegyptiaca* (Weber and Sotelo Montes 2010) in Niger, and in *P. biglobosa* in Burkina Faso (Ouedraogo et al. 2012). Billand and De Framond (1993) linked provenance variation of *F. albida* grown in Burkina Faso with the latitude, and promoted selected Sahelian provenances from Burkina Faso, Niger and Mali for reforestation programs. In *A. senegal*, Ræbild et al. (2003a,b,c) found large variation in growth and survival among provenances growing in different trials in Burkina Faso with Sahelian provenances (Burkina Faso, Mali and Niger) being superior to Sudanian provenances, and highlighted the weak performance of a provenance from India. In Sudan, Raddad and Luukkanen (2006) found genetic variation in growth, carbon isotope composition and gum production between *A. senegal* provenances from clayey and sandy soil areas of the gum belt in Sudan, and Raddad (2007) reinforced the differences by demonstrating genetic variation in seed morphology and seedlings traits between both type of provenances. Larwanou et al. (2010) assessed a mature *A. senegal* provenance trial in Niger, consisting of 11 provenances from Niger, Mali and Sudan and found a better performance of provenances from Mali and Niger in survival, height growth and basal area that correlated with the rainfall and geographical distances of the origins. In general, these clinal patterns suggest local adaptation and have implications for breeding and planting programs.

Few, if any, studies have compared how different environments influence the variation in performance among Sudano-Sahelian tree populations. Based on results from other regions, G×E interactions can be expected also for Sudano-Sahelian trees and may indeed be pronounced in the region due to the harsh growing conditions where poor adaptation instantly can generate high mortality and thereby trigger strong selection. In addition, substantial differences in climate, soil type and mineralogy are present within the natural distribution area of many Sudano-Sahelian tree species across Africa. Here, we study the situation for *Acacia senegal*.

Acacia senegal (L.) Willd. (Fabaceae, sub-family Mimosoideae) (syn. *Senegalia senegal* (L.) Britton) has a wide distribution across Africa (Sahelian belt and southern Africa) (Fagg and Allison 2004) and is also found in India and Pakistan. It is confined to arid zones and usually occurs in areas with low soil fertility and rainfall varying between 200-800 mm (Fagg and Allison 2004). The species includes four varieties namely vars. *senegal*, *kerensis*, *rostrata* and *leiorhachis*, where the variety *senegal* (the only variety present in Senegal) is the main gum arabic producing tree species in Sudano-Sahelian regions (Fagg and Allison 2004, Raddad and Luukkanen 2006, Diallo et al. 2015). *Acacia senegal* is an important source of income for rural populations that collect the gum Arabic. The species provides fodder particularly during dry periods, is used as fuel wood, restores soil fertility by its ability to fix nitrogen (Raddad et al. 2005), and is thus an essential component of dryland agroforestry systems (Fagg and Allison 2004, Raddad and Luukkanen 2006). Multipurpose tree species such as *A. senegal* are highly recommended in reforestation and agroforestry programs in the Sahel due to their socio-economic importance and their ability to tolerate periods of water deficit.

The present study aimed to investigate the performance and genotype-by-environment (G×E) interaction in survival and growth of African *A. senegal* provenances based on the analysis of two provenance trials in Senegal. We hypothesized that performance of provenances reflected adaptation to the climate at their site of origin, and that these differences in adaptation lead to G×E interactions when grown at sites with different levels of water stress. We analyzed whether provenance choice may be affected by the age at assessment and discussed the implications of our results in relation to future *A. senegal* breeding programs and management of genetic resources.

2. Materials and methods

2.1. Provenances and sites

The study includes two common garden trials established in August 1994 in Dahra (15º 20'N, 15º 28' W, elevation 45m) and Bambey (14º 71'N, 16º 47 'W, elevation 20m), Senegal. The trial site at Dahra is characterized by very dry growing conditions with an annual rainfall of 388 mm and an annual mean temperature of 28.1 ºC (estimates of WorldClim 2 2017). The soil is sandy, and the natural vegetation consists mainly of grass and sparse trees such as *Acacia tortilis* subsp. *raddiana* (Forssk.) Hayne (syn. *Vachellia tortilis* ssp. *raddiana* (Savi) Kyal. and Boatwr.), *A. senegal* and *Balanites aegyptiaca* (Göttsche et al. 2016). The trial site at Bambey is more humid with an annual rainfall of 482 mm and a mean annual temperature of 27 ºC (estimates of WorldClim 2 2017). The soil at Bambey is composed of leached tropical ferruginous sandy clays (Gray et al. 2013). The trials were protected against browsing by cattle and wild animals. Gum production was evaluated on the trees in 2001 and between 2007-2009 (Gray et al. 2013) by wounding the bark on selected branches. As all trees were harvested, we assume that the effect would have been similar on all trees.

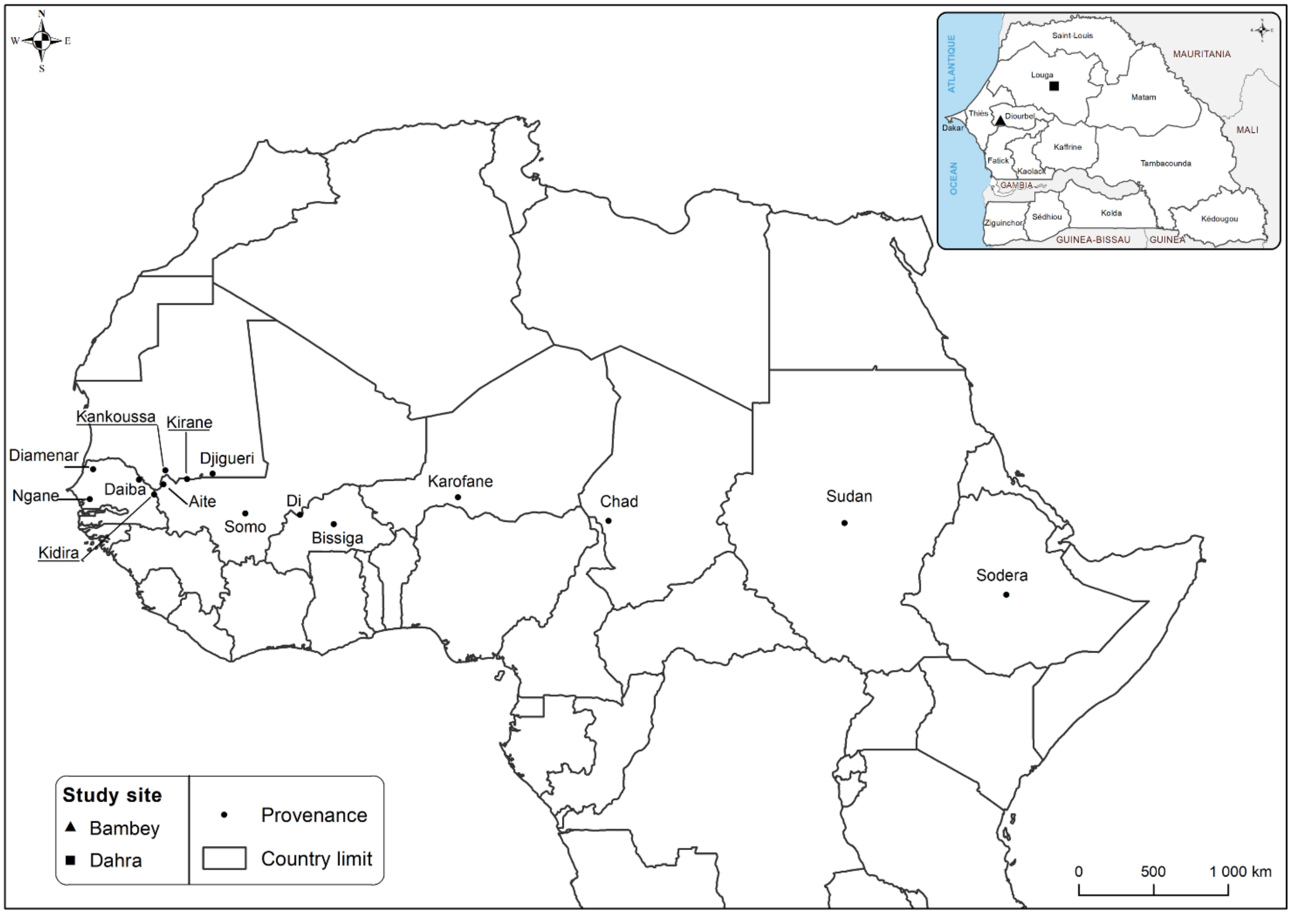

Fig. 1 Distribution the 15 African provenances of *A. senegal* located in the common gardens at Dahra and Bambey, Senegal. Insert in the upper right shows the location of the two trial sites in Senegal.

The provenance trials were established from seeds collected from 15 provenances across the distribution area of *A. senegal* in Africa (Fig. 1; Table 1). Both trials are randomized complete block designs with four blocks. Each block is divided into 15 plots, representing the 15 provenances originally with 25 trees each and a spacing of 5 x 5 meters i.e., 1500 trees at the time of establishment. The mean annual temperatures and the annual rainfall at the sites of origin of provenances were derived from the WorldClim2 database (Fick and Hijmans 2017). In addition, three aridity indices were used to describe the aridity conditions at the sites of origin, i.e. (i) De Martonne aridity index (A_M) which is the ratio between the mean annual rainfall (P) and temperature (T) plus 10 ºC (De Martonne, 1926); (ii) Thornthwaite aridity index (A_{TH}) calculated as the yearly sum of monthly ratios between rainfall (P) and evapotranpiration (P/E) (Thornthwaite 1931); and (iii) the monthly water availability indices (WAI) that were estimated as the differences between estimates of rainfall and potential evapotranspiration. These estimates were obtained from the Climatic Research Unit (CRU) East Anglia University version 4.01 database (Harris et al. 2014) for the period 1961-1990. We expect that climate differences among provenance sites for this period reflect the long-term differences in growing conditions that can have led to divergent natural selection in adaptive traits among provenances.

Yearly cumulated WAI (WAI0) was calculated as the sum of monthly WAI for the months where WAI was above zero (0) and provide an estimate of the water surplus available over the rainy season. Based on the yearly estimates the average was calculated for the period 1961-1990. Provenances with high WAI0 thus come from wet sites and provenances with low WAI0 come from dry sites.

Table 1 Origins and environmental characteristics of *A. senegal* provenances used in the study. Provenances are arranged according to WAI0.

Provenance	Country	Latitude	Longitude	Altitude (masl)	Mean annual temperature (ºC)^{a}	Rainfall (mm)^{a}	PET (mm)^{b}	Martonne index (A_{M})^{b}	Thornwaite index (A_{TH})^{b}	WAI0 (mm)^{b}
Diamenar	Senegal	16º00’ N	15º54’ W	20	27.13	288	1950	8	2	28
Djigueri	Mauritania	15º44’ N	08º40’ W	226	28.92	309	2195	8	2	45
Karofane	Niger	14º18’ N	06º11’ E	280	28.38	366	2412	10	2	51
Sudan	Sudan	12º44’ N	29º35’ E	620	26.84	408	1775	11	3	56
Kirane	Mali	15º23’ N	10º15’ W	140	29.32	380	2018	10	3	59
Kankoussa	Mauritania	15º56’ N	11º27’ W	80	29.71	294	1917	7	3	60
Chad	Chad	12º49’ N	15º18’ E	280	28.97	349	2098	9	3	64
Daiba	Senegal	15º22’ N	13º08’ W	28	29.56	437	1813	11	3	65
Aite	Mali	15º05’ N	11º39’ W	80	29.65	449	1855	11	3	75
Di	Burkina Faso	13º10’ N	03º25’ W	260	28.02	637	1832	17	5	121
Bissiga	Burkina Faso	12º26’ N	00º32’ W	280	28.28	696	1915	18	5	128
Kidira	Senegal	14º28’ N	12º13’ W	39	28.93	611	1724	16	5	184
Somo	Mali	13º17’ N	04º54’ W	40	27.45	627	1767	17	6	187
Ngane	Senegal	14º08’ N	16º12’ W	2	27.92	570	1871	15	5	194
Sodera	Ehiopia	08º24’ N	39º23’ E	1500	21.36	762	1302	24	10	292
Sites										
Dahra	Senegal	15º 20’N	15º 28’ W	45m	28.1	388	1805	10	3	75
Bambey	Senegal	14º 71’N	16º 47’ W	20m	27	482	1864	13	4	122

^{a} Climate data derived from WorldClim, ^{b} estimates based on climate data from the CRU East Anglia University version 4.01 database (Harris et al. 2014). De Martonne Aridity index (A_{M} = P/(T + 10), Thornthwaite aridity index (A_{TH} = yearly sum of monthly ratios (P/E)), cumulated water availability index (WAI0) for the months with WAI > 0, mean for the years 1961-1990.

2.2. Data collection and analysis

The growth was assessed in 2008 i.e., 14 years after establishment in both common garden trials. The height of the trees was measured using a telescopic measuring pole, and the stem diameter was measured at 30 cm (basal diameter) above ground level using a caliper. For the estimation of survival, a record of (0) was given for a dead tree, and (1) for a living tree. For the Dahra trial, the same measurements were also made in 2017, i.e. at age 23 but similar data could not be obtained from Bambey.

The analysis of variance was based on average values per plot to assure independence between residuals. The percent of living trees in each plot was calculated and used for the analysis on survival. The R statistical package software (R Core Team, 2020) was used for all analysis, and figures were produced using the packages *ggplot2* (Wickham 2016) and *ggpubr* (Kassambara 2020).

Analyses were based on three steps.

1) Quantifying and testing differences among the *A. senegal* provenances when grown at each of the two test sites. This step was used to calculate the average performance of the provenances at each of the two sites Bambey (age 14) and Dahra (ages 14 and 23). To study the effect of assessment age, we tested the relative performance between years, and calculated the correlation between age 14 and age 23 for the Dahra trial where data from both years were available.
2) Comparing the performance of the provenances at each of the two sites with the climate at their site of origin based on linear regression. The step allowed us to study the extent to which the differences among the provenances in their survival and growth (height and diameter) can be explained by matches between the climatic conditions at the test site, and at the origin of the provenances, and to what extent such patterns differed between the two test sites.
3) Testing if the relative performance of provenances depends on the site and age. To obtain scale free measures of the populations x site interaction, we estimated correlations between provenance least square means for each site (similarly to the approach suggested by Burdon (1977)). To investigate the potential underlying causes of the interactions, we estimated how much provenance height and diameter (as proxies for growth) and survival were improved at the wetter Bambey site compared to the drier Dahra site, and used linear regression models to test if these differences depended on climate at the sites of origin.

For the tests of variation among provenances, years and G×E interaction in survival, height and diameter (Steps 1 and 3), we applied the three general linear models (1), (2) and (3) (for analysis per site and across years and sites, respectively) using the function *lmer* (Linear Mixed-Effect Models) in the package *lme4* (Linear Mixed-Effect Models using "Eigen" and S4) (Bates et al. 2015) for R (version 3.4.2):

$$Y_{ij} = \mu + B_i + P_j + \varepsilon_{ij}, \tag{1}$$

Where Y_{ij} is the plot average of the trait in block i. B_i is the random effect of block i, P_j is the fixed effect of provenance j, and ε_{ij} is the residual error assumed to be independent and following a normal distribution.

$$Y_{ijk} = \mu + B_{i(k)} + P_j + Z_k + PZ_{jk} + \varepsilon_{ijk} \tag{2}$$

Where $Y_{ij(k)}$ is the plot average of the trait in the block i at the site k. $B_{i(k)}$ is the random effect of the block i within site k, P_j is the fixed effect of provenance j, Z_k is the fixed effect of the site k, PZ_{jk} the random interaction of the provenance j and site k, and ε_{ijk} is the residual error assumed to be independent and following a normal distribution. Residuals were plotted against fitted values and by sites to control for presence of heteroscedasticity, and residual histograms were plotted to check the assumption of normal distribution. Least square means were estimated for the provenances.

A model (3) similar to model (2), but with years replacing sites, was used to test for effects of years and effects of provenance by year interactions at Dahra.

Regression analyses were applied to study relationships between provenance performance on the one side and climate at the origin of the provenance and latitude, longitude, and altitude of the provenance origin on the other side (Step 2). The function *corr.test* in the package *psych* (Revelle 2019) was used to test for significance of correlations.

Regression analyses and plots were made using the *ggscatter* function in the package *ggpubr* (Kassambara 2020) as implemented in R (R Core Team 2020).

For step 3, we first calculated the relative performance of each provenance $Z_{j(k)}$ at each site ($Z_{j(k)} = (X_{j(k)} - \mu_k)/ \mu_k)$) where $X_{j(k)}$ is the least square mean estimate for provenance j at site k and μ_k is the average of least square mean estimates at site k. $Z_{j(k)}$ values above 0 thus reflected above average performance of a specific provenance at the site, while negative values reflected the opposite. We then calculated the difference between the two sites in the value $G_j = Z_j(\text{Bambey}) - Z_j(\text{Dahra})$. $G_j > 0$ thus reflects that provenance j perform relatively better at Bambey while $G_j < 0$ reflects that provenance j perform relatively better at Dahra. We tested the regression of G_j on annual rainfall and water availability index (WAI0) at the provenance origins using the *ggscatter* function. Pearson's correlations between sites were estimated based on least square means of provenances for each site.

3. Results

3.1. Survival, height and diameter

Most provenances had the highest survival and largest height and diameter at the wet site Bambey, compared to the dry site Dahra (Table 2). At age 14, the overall mean survival rate in Bambey was 58%. The overall mean height and diameter were 4.8 m and 11.6 cm, respectively, compared to only an overall mean survival rate of 25% at Dahra and overall mean height and diameter of 4 m and 8.6 cm, respectively (Table 2). At age 23, the overall mean survival at Dahra was decreased to 18% while the overall mean diameter was increased to 14.4 cm (Table 2).

Table 2 Least square means for survival rate, height and diameter of *Acacia senegal* provenances in Bambey and Dahra (model 1). Provenances are arranged according to WAI.

Provenance	Survival (%)			Height(m)			Diameter (cm)		
	Bambey	Dahra	Dahra	Bambey	Dahra	Dahra	Bambey	Dahra	Dahra
	Age 14	Age 14	Age 23	Age 14	Age 14	Age 23	Age 14	Age 14	Age 23
Diamenar	56	36	29	4.6	3.8	4.0	10.2	7.9	13.2
Djigueri	72	27	26	4.6	4.5	4.2	10.3	9.2	15.6
Karofane	69	24	22	4.3	3.7	3.9	11.0	8.4	13.2
Sudan	72	44	20	4.8	4.5	4.4	11.4	9.6	13.9
Kirane	66	25	18	4.8	4.2	4.3	11.1	9.0	14.1
Kankoussa	49	33	29	4.2	3.4	4.0	9.9	6.6	13.0
Chad	85	28	16	5.3	4.8	4.6	14.5	10.4	18.6
Daïba	60	26	18	4.4	4.4	4.4	9.4	10.2	14.9
Aite	47	25	20	5.7	4.5	4.7	13.4	8.8	15.1
Di	30	13	3	3.9	3.8	4.1	9.9	8.6	15.6
Bissiga	62	11	9	5.2	4.3	5.0	13.3	10.0	18.9
Kidira	59	23	15	5.5	3.8	4.2	12.4	7.4	11.6
Somo	41	14	8	4.5	2.8	3.7	11.4	5.9	12.8
Ngane	62	21	17	5.8	4.5	4.7	15.2	9.8	16.8
Sodera	38	18	12	4.0	3.3	3.3	10.3	7.4	9.1
p value	0.0031**	0.0055**	0.02136*	< 0.0001***	0.1376	< 0.0001***	< 0.0001***	0.0451*	0.0003***
mean	57.9	24.5	17.5	4.8	4.0	4.2	11.6	8.6	14.4
S.E	10.8	5.2	4.7	0.2	0.5	0.2	0.5	1.0	1.2

S.E. standard error of provenance least square means

In Bambey, the survival rate, height and diameter varied significantly among provenances (Table 2). The provenance Chad had the highest survival rate (85%) while the provenance Di (Burkina Faso) had the lowest value (30%). With respect to growth, the local Senegalese Ngane provenance was superior in both height and diameter, but there were also slow growing Senegalese provenances, e.g. Daiba that had the smallest diameter of all provenances at this site (Table 2).

In Dahra, differences among provenances were significant except for height at age 14. Here, the span in survival at age 14 varied from 44% (Sudan) to 11% (Bissiga from Burkina Faso). For diameter, the provenance Chad had the largest diameter (10.4 cm), but based on few trees because of low survival, while Somo from Mali had the smallest (5.9 cm) diameter and also low survival, (Table 2). At age 23, the span in survival was from 3% to 29% with Bissiga and Somo below 10% (Table 2). The interactions between provenance and year in Dahra were not significant for neither survival, height or diameter (Table 3), and the age-age correlations between age 14 and 23 correspondingly were high for both diameter ($r_{(14;23)} = 0.75$) and survival ($r_{(14;23)} = 0.78$) (Fig. 3a,b). The correlations between survival and diameter were non-significant for both age 14 ($r_{(14)} = -0.17$) and age 23 ($r_{(23)} = -0.14$).

Table 3 Analysis of variance for *A. senegal* at Dahra in 2008 (age 14) and 2017 (age 23) (model 2)

Source	**df**	**SS**	**MS**	**F**	**P**
Survival rate					
Provenance	14	0.5702	0.0407	4.11	< 0.0001***
Year	1	0.1359	0.1359	13.72	0.0004**
Provenance × Year	14	0.0857	0.0061	0.62	0.8431
Height					
Provenance	14	23.2206	1.6586	3.47	0.0002***
Year	1	0.1605	0.1605	0.34	0.58300
Provenance × Year	14	2.8037	0.2003	0.42	0.96440
Diameter					
Provenance	14	336.62	24.044	5.56	< 0.0001***
Year	1	250.22	250.221	57.86	< 0.0001***
Provenance × Year	14	76.75	5.482	1.27	0.2464

3.2. Relationship between survival and climate at the site of origin

The regression analyses identified significant relationships between provenance survival at the two sites and annual rainfall at the site of origin (Fig. 2). Generally, provenances from drier areas survived better at both sites, but the relationship was strongest at the driest test site (Dahra). No significant relationship was found between diameter or height and any of the climate variables. This was the case at both sites (not shown).

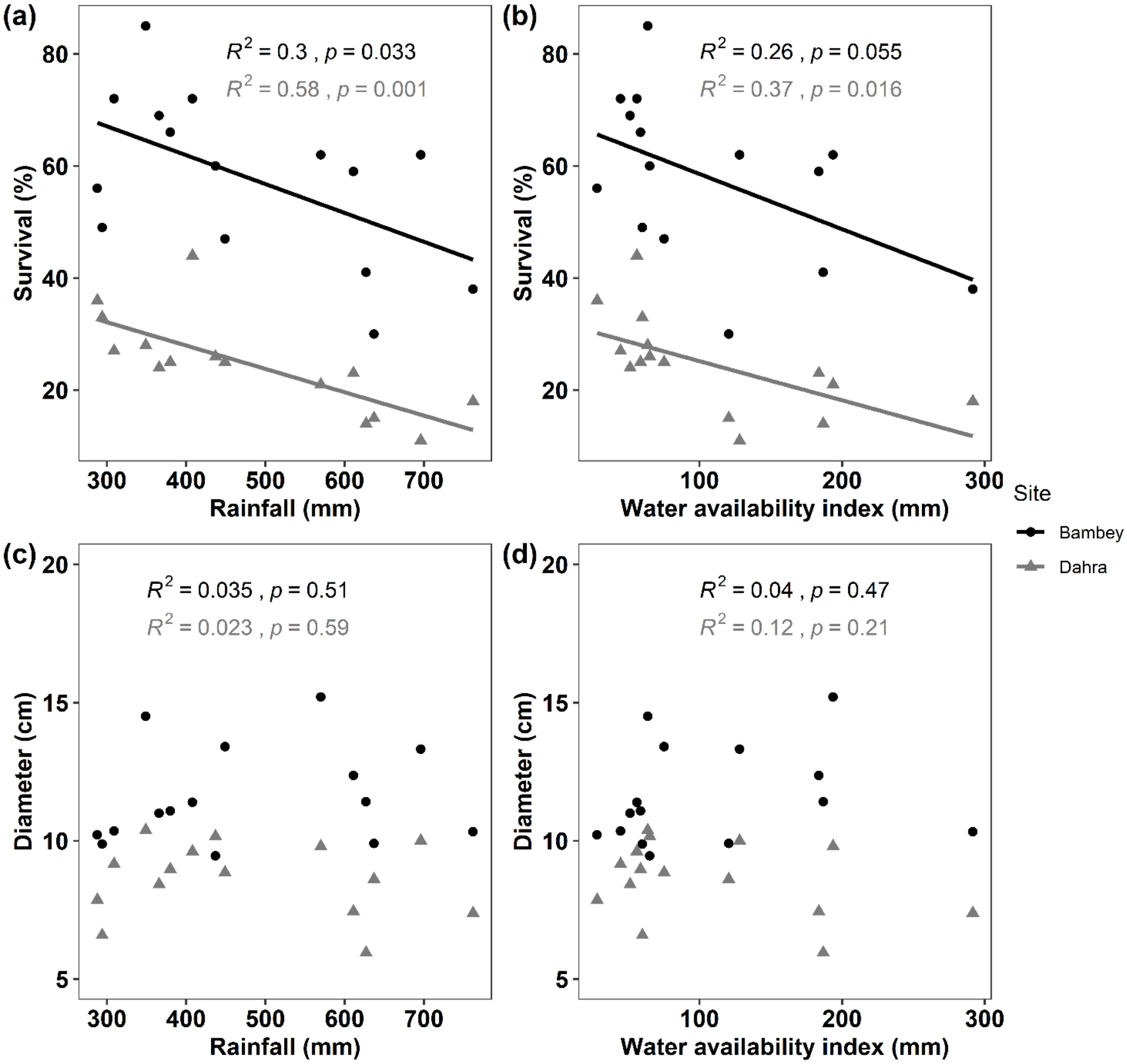

Fig. 2 Pair-wise plots of survival rate and diameter against annual rainfall (a, c) and the cumulated water availability index for the months with precipitation > potential evapotranspiration (WAI0) at Dahra and Bambey (b, d). R^2 and P-values from regression analyses are inserted.

3.3. Genotype x environment interaction

The interaction between provenances and sites (G×E) was highly significant for diameter, but only close to significance for survival and not significant for height (Table 4). Correspondingly, the Pearson's correlation between provenance least square means for diameter at the two sites was only moderate (r = 0.41) with changes in rank between the two sites (Fig. 3c).

Table 4 Analysis of variance for *A. senegal* at Dahra and Bambey (model 3)

Source	**Df**	**SS**	**MS**	**F**	**P**
Survival rate					
Provenance	14	1.12	0.08	3.93	< 0.0001***
Sites	1	0.46	0.46	22.53	0.0031**
Provenance × Site	14; 1	0.47	0.03	1.66	0.0802
Height					
Provenance	14	29.64	2.12	4.91	< 0.0001***
Sites	1	2.36	2.36	5.46	0.0579
Provenance × Site	14; 1	7.50	0.54	1.24	0.263
Diameter					
Provenance	14	195.56	13.97	6.53	< 0.0001***
Sites	1	97.30	97.30	45.51	0.0005***
Provenance × Site	14; 1	77.97	5.57	2.60	0.0036**

As illustrated in Fig. 3c, the Senegalese provenance Kidira had a faster diameter growth (7% above the average) at Bambey, but a poorer growth at Dahra (-14%). Provenances Sudan (Sudan), Kirane (Mali), Djigueri (Mauritania) and Daiba (Senegal) performed better than the average at Dahra (12%, 4%, 6%, and 18% respectively) and poorer in Bambey (-2%, -4%, -10%, and -18% respectively). The four provenances Ngane (Senegal), Chad, Aite (Mali) and Bissiga (Burkina Faso) had above average performance at both sites, while Somo (Mali), Karofane (Niger), Diamenar (Senegal), Di (Burkina Faso), Sodera (Ethiopia) and Kankoussa (Mauritania) performed poorly at both sites.

G×E for survival was close to significance (P=0.08; Table 4) and we observed some change in rank among provenances when plotting provenances' performance at the two sites (Fig. 3d). The Pearson's correlation between provenance least square means for survival at the two sites was also only moderate (r = 0.47).

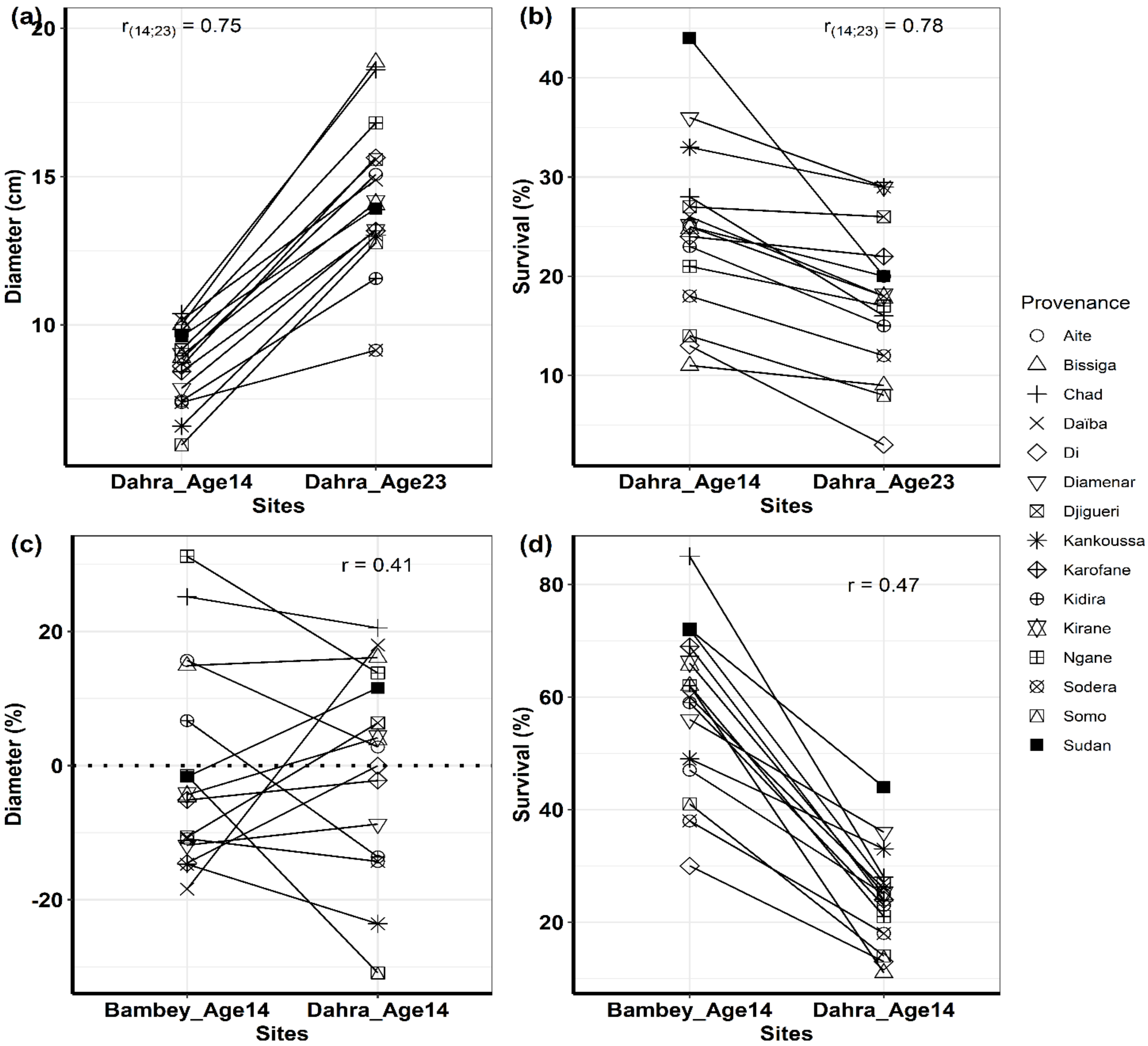

Fig. 3 Performance of *A. senegal* provenances between years (Dahra 2008 and Dahra 2017) and across sites (Dahra and Bambey 2008). Fig. 3 a, b and d are least square means values of the provenances and Fig. 3 c is Z, % deviation from the mean at the trial site). Note: G x E interaction for survival was not significant (P = 0.08), r: Pearson's correlation coefficient.

3.4. Relationship between difference in provenance performance at the two sites and climate at their origin

The difference in relative performance in height and diameter of the provenances at the two sites (G_j) was significantly related to water availability index at the provenance sites of origin. Provenances from areas with higher water availability thus ranked relatively better in terms of height and diameter at the wetter site Bambey, compared to drier site Dahra (Fig. 4). Relationships with other climate variables, i.e. mean annual rainfall, temperature, and the A_M and A_{TH} aridity indices, were not significant (Table S1). For survival, none of the regressions of G_j on annual rainfall, mean annual temperature and aridity indices were significant.

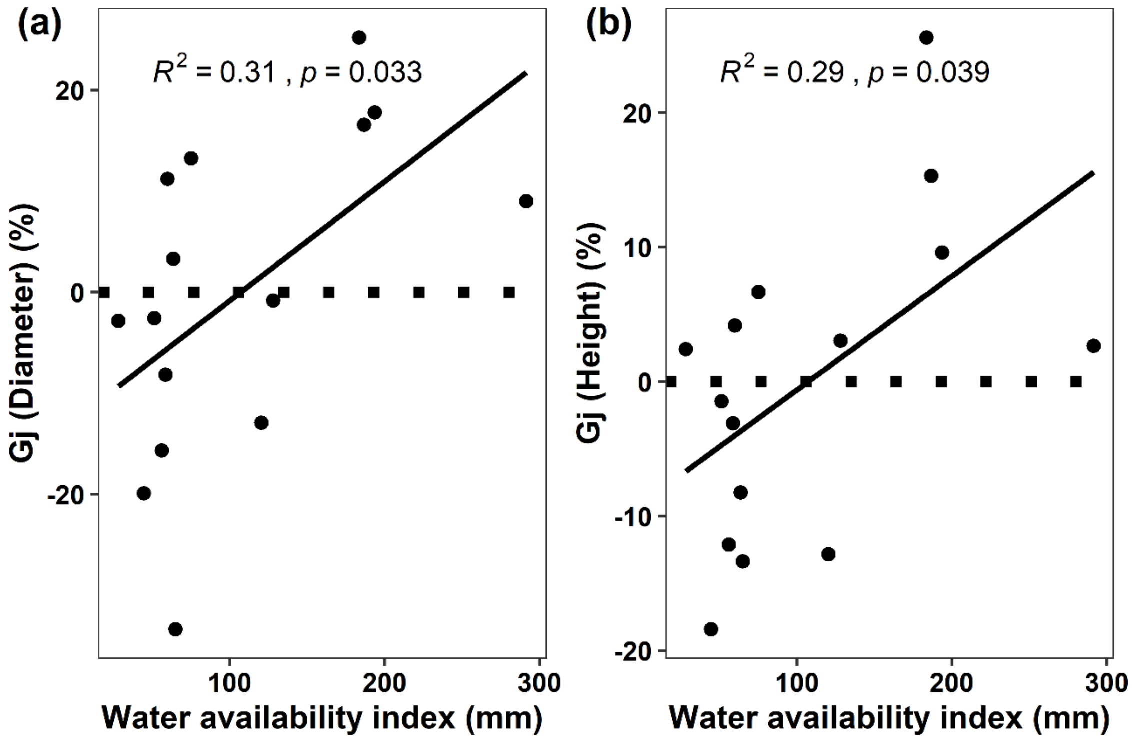

Fig. 4 The relative advantage of growth at the wetter site Bambey compared to the dry site Dahra (Gj) of the provenances related to the cumulated water availability index for the months with precipitation > potential evapotranspiration (WAI0) at the sites of origin of the provenances. Values above 0 denote relative superiority at the wet site Bambey and values below 0 denote relative superiority at the dry site Dahra.

4. Discussion

4.1. Variation among provenances and sites

The present study is based on results for only two trials in one country and the extrapolation of the results should be done with caution. However, the study demonstrated that survival and growth (height and diameter) of *A. senegal* trees depended on their genetic origin, and that the observed variation among the provenances in survival could be partly explained by the climatic conditions at their sites of origin. Trees that originated from dry sites in general survived better than trees from wetter sites at both test sites. However, with respect to height and diameter, the provenances from wetter sites generally ranked better at the wet test site (Bambey) while provenances from drier sites in general ranked better at the dry site (Dahra). These results support that the observed variation among provenances in their survival and growth reflects adaptation to local climatic conditions. Such development of ecotypes reflects a general trend to differentiation in physiological and morphological traits as response to divergent natural selection (Eriksson et al. 2020).

The significant variation among provenances confirmed the genetic differences in growth traits among natural populations of *A. senegal* when tested in common garden trials in Africa (Ræbild et al. 2003a,b,c; Raddad and Luukkanen 2006; Raddad 2007; Larwanou et al. 2010). Genetic differentiation in growth among provenances tends to be a common feature of Sahelian tree species (Weber and Sotelo Montes 2010; Chládová et al. 2019; Lompo et al. 2020). Genetic variation among origins of *A. senegal* has also been documented based on molecular markers (Assoumane et al. 2012, Odee et al. 2015, Diallo et al. 2015). DNA based studies have revealed that many natural populations of *A. senegal* consist of a mixture of diploid and polyploid trees, where the ploidy level can influence the fitness of the trees (Diallo et al. 2016). We have in a parallel study found that the provenances analysed in the present study differ in frequency of polyploids, and documented morphological differences that may reflect adaptation to different environments (Diatta et al. 2021).

From an applied perspective, the variation among provenances allows identification and use of productive provenances (in terms of growth) in restoration and tree improvement programs of *A. senegal*. However, the present study documents presence of G×E interaction, and selection of superior provenances for planting programs must therefore be based on testing at multiple sites in order to be able to reveal the best genetic origin for a specific site. This is a common situation in many species (Wu and Matheson 2005; Correia et al. 2009; Rweyongeza 2011; Belaber et al. 2020), but here shown for the first time in *A. senegal*. Our study is based on only two sites, and can therefore only be seen as a pilot study when it comes to mapping G×E. A more fine scaled set of test sites will be required to guide number and location of test sites for breeding of *Acacia senegal*. However, an alternative to classical breeding programs with multiple test sites can be to accommodate the presence of G×E by applying domestication based on a decentralized, multiple breeding concept (see Namkoong et al. 1980; Dhakal et al. 2005).

Previous studies have suggested presence of local adaptation in *A. senegal*. When investigating genetic variation among African provenances, Larwanou et al. (2010) found that performance varied clinally with rainfall while Ræbild et al. (2003a,b,c) demonstrated that local West African Sahelian provenances performed better than Sudanian provenances in trials in Burkina Faso. Raddad (2007) highlighted the formation of ecotypes differentiated by morphological and growth parameters in young plants. The G×E interactions presented here based on a reasonably high number of provenances confirm local adaption to rainfall with respect to growth parameters. Still, the present study is only based on two sites. Future investigations based on establishment of a larger number of trials across Sahel based on an internationally coordinated effort will be highly valuable.

Clinal variation in survival have previously been observed in provenances of *Prunus africana* in Niger (Weber et al. 2008; Sotelo Montes and Weber 2009; Weber and Sotelo Montes 2010). It was demonstrated that growth and survival of *P. africana* increase from more humid to drier parts of the sample region. This is in line with our observed clines in survival that likely reflect a local adaptation of *A. senegal* to drought. The ecophysiological background of the observed clines remains unknown, but may be due to differences in rooting depth and the ability of roots to withstand drought stress and the maintenance of physiological functions (Poorter et al. 2012; Olmo et al. 2014; Brunner et al. 2015). *Acacia senegal* like many Sahelian species is a drought tolerant species with an ability to use water and nutrients efficiently, and a relatively large allocation of biomass to roots (Raddad and Luukkanen 2006; Raddad 2007; Gray et al. 2013; Merine et al. 2014). A general feature of tree species adapted to dry environments is that they develop higher root-to-shoot ratios and deeper root systems compared to species from mesic environments (Markesteijn and Poorter 2009; Hartmann 2011; Brunner et al. 2015). Differences in rooting depth and the ability of plants to access soil moisture at depth are likely to influence plant survival (Padilla and Pugnaire 2007; Poorter et al. 2012; Olmo et al. 2014). The initial growth in *A. senegal* is predominantly underground with seedlings developing a long tap-root (Fagg and Allison 2004), and in a provenance trial in Sudan, seedlings of *A. senegal* provenances from sandy soils had longer root lengths compared to provenances from clayey soils (Raddad 2007). Generally, as a response to drought, plants tend to decrease shoot biomass and increase root biomass, allowing them to reduce water loss by transpiration and increase the efficiency of soil exploration and water acquisition, leading to a higher probability of survival (Lloret et al. 1999; Poorter et al. 2012). Based on the findings of the present study, it will therefore be interesting to test if *A. senegal* provenances from the dry sites develop deeper rooting systems compared to provenances from wetter sites.

4.2. Ecology and implications of the G×E interaction

While data indicate that *A. senegal* provenances from the drier parts of the distribution area in Africa are superior with respect to survival, provenances from the wetter parts show superior performance with respect to above-ground growth under favorable (less dry) conditions. This suggests that while survival is the most critical for fitness under dry conditions, it may come with a negative fitness trade-off against fast growth (which may increase competitiveness for other resources, and options for earlier and more abundant seed production) under more humid conditions.

The relative advantages of different *A. senegal* provenances to grow under either wet or dry conditions may reflect different water-use strategies (Raddad and Luukkanen 2006; Gray et al. 2013; Li and Wang 2003) as also discussed above. The WAI0 is higher at Bambey (122 mm) compared to Dahra (75 mm). In addition, the sandy-clay soils at Bambey is likely to have higher water holding capacity compared to the sandy soils at Dahra and therefore less prone to desiccation in the growing season. Studies of stable isotopes suggested variation among *A. senegal* provenances in their water use efficiency (Raddad and Luukkanen 2006; Gray et al. 2013; Sarr et al. 2021; Diatta et al. 2021). For example, *A. senegal* provenances from clay soils displayed a less conservative water use efficiency (WUE) resulting in fast growth and high gum productivity compared with provenances from sandy soils that displayed more conservative water use (Raddad and Luukkanen 2006). Similar results on variation on WUE were found in *A. tortilis* ssp. *raddiana* at three semi-arid sites in Kenya (Newton et al. 1996), in dominant woody species on a moisture gradient in an African savanna in Bostwana (Midgley et al. 2004), and in dominant species along a continental-scale climate gradient in Australia (Rumman et al. 2018). However, Gray et al. 2013 found that seedlings and mature *A. senegal* trees may exhibit different WUE strategies suggesting plasticity in WUE that optimizes carbon assimilation and water use at young age while plants are shallow-rooted, but relax water control at an advanced age when roots are deeper and able to access groundwater. Indeed, the root system of adult *A. senegal* trees represents 40% of the total biomass compared to the aboveground biomass, i.e. stem and branches (Poupon 1977 in Fagg and Allison 2004). Whether our provenances from wetter sites exhibited a prodigal water use strategy that led to a faster diameter and height growth but lower chances of survival will have to be tested in follow up experiments.

4.3. Choice of provenances for planting activities

Regarding selection of future germplasm for breeding, the provenances Sudan, Kirane, Djigueri and Daiba that had largest diameter at Dahra, and originate from areas with WAI0s close to the WAI0 of the trial site. According to Gray et al. (2013) the Sudanese provenance have high gum production. As Bambey is located outside the zone of gum production in Senegal (Gray et al. 2013), we suggest to prioritize the best provenances at Dahra when planning selection for gum production, because we consider this site the better representative of sites suitable for plantings of *A. senegal* for gum Arabic production. However, the observed G×E calls for caution against providing general recommendation based on the trial when planting the species under environmental conditions that differs from the trial site in Dahra. As discussed above, working in multiple breeding zones based on selection of genotypes from origins having similar environmental conditions to the site of planting is therefore advisable.

The comparison of survival and growth after 14 and 23 years at Dahra showed that the main patterns in general did not change much during the 9 years. An interesting exception is the provenance from Sudan that had highest survival and good growth at the Senegal trial after 14 years, but has lost its superiority when assessed at age 23. The general provenance-by-age interaction was not significant, but the changed rank of the Sudan provenance draws attention to the fact that especially exotic provenances should be tested for longer periods before it can be concluded that they are superior to native origins.

5. Conclusion

The present study supports that African provenances of *A. senegal* are genetically differentiated in important adaptive traits as result of local adaption to different ecological conditions within the species' natural distribution in Africa. The results point towards the need for development of seed transfer guidelines and policies to avoid planting trees with poor adaptation in planting programs. This implies that breeding programs should carefully identify breeding and deployment zones. The results also highlight the importance of conserving genetic resources of the economically very valuable species based on a network of multiple populations in order to sample variation among populations. This is crucial in the face of ongoing climatic change where todays arid sites may become important seed sources for tomorrow’s plantings programs in desertificated areas.

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Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

All authors gave their informed consent to this publication and its content.

Availability of data and material

Data is publicly available through the University of Copenhagen - Electronic Research Data Archive (ERDA).

Authors' contribution

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Supplementary material

Table S1. Pairwise correlations between relative advantage of growth (height and diameter) and survival at the wetter site Bambey compared to the dry site Dahra (*Gj*) of populations means and climate variables at their sites of origin.

	G_j (height)	G_j (Diameter)_	G_j (Survival)	Latitude	Longitude	Altitude	Rainfall	Mean_temp	A_M	A_{TH}	WAI0
G_j (Height)	-	0.89***	0.08	-0.05	-0.25	-0.11	0.40	-0.07	0.36	0.40	**0.54***
G_j (Diameter)		-	0.08	-0.18	-0.07	0.02	0.35	-0.14	0.33	0.41	**0.55***
G_j (Survival)			-	-0.20	-0.07	0.01	0.41	0.13	0.32	0.20	0.27
Latitude				-	-0.84**	-0.9***	-0.72**	0.84***	-0.81***	-0.84***	-0.73**
Longitude					-	0.88***	0.27	-0.72**	0.4	0.48	0.31
Altitude						-	0.48	-0.89***	0.64*	0.72**	0.56*
Rainfall							-	-0.53*	0.98***	0.9***	0.88***
Mean_temp								-	-0.69**	-0.76***	-0.67**
A_M									-	0.96***	0.920***
A_{TH}										-	0.96***
WAI0											-

Mean_temp: annual mean temperature, A_M: De Martonne aridity index (P/(T + 10)), A_{TH}: Thornthwaite aridity index (yearly sum of monthly ratios (P/E)), WAI0: cumulated water availability index for the months with WAI > 0, mean for the years 1961-1990.

Manuscript III. Variation in phenology of *A. senegal* (L.) Wild. in relation to origin and ploidy level - implications for climatic adaptation

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Abstract

Heritable phenotypic variation in fitness traits is essential for species' response to environmental changes through natural selection. This can be of special importance in areas with long dry seasons where growth is confined to the wet season. However, knowledge of variation in phenology of deciduous African dryland species such as *Acacia senegal* is limited. This study therefore investigated variation in phenology within and among populations of *A. senegal* trees growing in a common garden trial in Senegal. The leafing, flowering and fruiting phenology were monitored for 17 months and compared to observations of the rainfall during the period. We found that *A. senegal* started development of leaves prior to the beginning of the raining season with flowering and fruiting initiation occurring during the raining season. We concluded that phenology in *A. senegal* is under genetic control, because significant differences could be observed among populations and ploidy levels when grown at the same site. In general, early leaf flushing trees had a longer growing period and performed better in terms of growth at the tested site. The results for diploid trees indicate that the leaf phenology reflected local adaptation, but the same patterns were not obvious for tetraploid trees. Flowering phenology differed between diploid and tetraploid trees, but not to an extent that suggests it could provide an effective reproductive barrier between the two ploidy levels. Our study suggests further investigations on environmental cues that can trigger leafing phenology, the associated physiological mechanisms, and the potential reproductive barriers between diploids and tetraploids individuals.

1. Introduction

Phenology refers to the timing of plant growth and reproduction (Rathcke and Lacey 1985), and is an important component of plant fitness in seasonal climates. Phenology is generally controlled by environmental cues such as temperature, photoperiod and water availability (Polgar et al. 2014; Omondi et al. 2016; Di Lucchio et al. 2018), and is therefore also a biological indicator of climate change (Parmesan 2006; Cleland et al. 2007; Nicotra et al. 2010). The acclimation of plant phenology to prevailing climatic seasonality enables plants to grow and reproduce under the climatic conditions at the given site and in the given year (Lim et al. 2007, Omondi et al. 2016).

Seasonal variation in temperature, humidity, rainfall, wind speed and although tropical ecosystems are not characterized by strong differences between summers and winters in photoperiod as seen at higher latitudes, even day length can control phenology in tropical plants (Lieberman 1982). In tropical drylands, water availability is generally the main environmental factor controlling phenology (Borchert 1994; Eamus and Prior 2001; Do et al. 2005). However, some tropical dryland species produce leaves and flowers before the onset of the raining season while others do so after the onset of the season (Lieberman 1982; Tybirk 1993). Observations made by satellite imagery of population stands revealed that leafing phenology of tropical dryland species may be triggered by other environmental cues than water availability (Ryan et al. 2017; Tian et al. 2018).

Perennial plants must time their growth to match the annual variation in temperature and water availability. The length of the growing season (time of leaf appearance to leaf senescence) defines the window for photosynthetic activity and therefore affects water, carbon and nutrient cycling and the net ecosystem production (Wu et al. 2013; Panchen et al. 2015), and too late or early leaf flushing or too late or early senescence will therefore reduce growth. The timing of flowering and fruiting can be similarly important for successful plant reproduction (Milla et al. 2005), because plants that flower too early, before having time to accumulate enough resources, can have a limited capacity for seed production, while plants that delay flowering may might gain higher capacity or might lack time to produce mature fruits before the end of the season (Omondi et al. 2016). Besides adaptation to prevailing seasonality, differences among species in phenology can also facilitate coexistence of species in diverse plant communities by reducing competition for pollinators and other resources (Rathcke and Lacey 1985; Khanduri, 2014).

Studies of variation within and among tree populations in their phenology can reveal the degree of local adaptation and phenotypic plasticity in response to variation in climate, and therefore shed important light on wise management of genetic resources in the face of climate change. There is limited knowledge of genetic variation in phenology of African dryland species (Ræbild et al. 2010). However, recent analyses of provenance trials (common garden trials) have revealed clear differentiation among populations of species that cover substantial ecological variation. Genetic variability in phenological traits has thus been reported in *Adansonia digitata* (Di Lucchio et al. 2018; Bamba et al. 2019) and *Parkia biglobosa* (Ouedraogo 2014). In a greenhouse trial, Di Lucchio et al. (2018) exposed *A. digitata* seedlings' of different origins to different water and day length treatments, and found that the leaf phenology depends on both day-length and water regimes, but also that the response varied among geographic origins. Bamba et al. (2019) reinforced the findings by exploring a provenance trial in Mali, and showed that *A. digitata* populations differed in their senescence, and that these differences could be partly by climate of the site of their origins as an indication of adaptation. In a provenance trial in Burkina Faso, Ouedraogo (2014) explored different populations of *P. biglobosa* and found that leaf phenology was correlated with the latitude and temperature of the origins with provenances from cooler areas reaching their flushing peak earlier than provenances from hotter areas.

Acacia senegal is a multipurpose tree species that is widely distributed across Africa from the Sahelian belt to southern Africa (Fagg and Allison 2004), and is also found in India and Pakistan. The species is recently conferred to the new name *Senegalia senegal* (L.) Britton (Kyalangalilwa et al. 2013), but here we maintain the rule of first priority and the name *A. senegal* is used in the present publication. *Acacia senegal* is well adapted to arid zones with low soil fertility and a seasonal rainfall varying between 200-800 mm (Fagg and Allison 2004). Gum Arabic is collected from the tree (Fagg and Allison 2004; Raddad and Luukkanen 2006; Diallo et al. 2015), and provides an important source of income for rural populations that also use the species for other products. The ability to fix nitrogen and tolerate drought impacts makes *A. senegal* an essential component of dryland agroforestry systems (Fagg and Allison 2004; Raddad et al. 2006) and the species is therefore widely recommended in reforestation programs in Sahel. A number of studies have investigated the phenological events of *A. senegal* (Tandon et al. 2001; Seghieri al. 2012b; Omondi et al. 2016), but the genetic variation among origins that can reflect adaptation to the climate conditions has to our knowledge not been described.

Genetic differentiation among origins of *A. senegal* has been found in growth (Ræbild et al. 2003a,b,c; Raddad and Luukkanen 2006; Raddad 2007; Larwanou et al. 2010; Diatta et al. 2021, Manuscript 2) and when investigating molecular markers (Assoumane et al. 2013; Odee et al. 2015; Diallo et al. 2015). The studies have revealed patterns of adaptation to local climatic conditions, and also that the species can co-occur in different levels of ploidy (different numbers of sets of chromosomes), where the level of ploidy influences the species' performance and response to drought stress (Diallo et al. 2015; Diallo et al. 2016; Diatta et al. 2021 (Manuscript 1)). It is therefore important to take the level of ploidy into account when studying the genetic variability in phenological traits of *A. senegal.*

The present study aims to investigate variation in phenology among different origins (populations) of *A. senegal*, and whether these differences can be related to the climate at the origin of the population or to the ploidy level, and whether there is a barrier in flower phenology, which inhibits crossings between diploid and tetraploid individuals.

2. Material and methods

The present study was conducted in a common garden trial established in August 1994 in Dahra (15º 20'N, 15º 28' W, elevation 45m) Senegal. The trial site is characterized by very dry growing conditions with an annual rainfall that ranged between 169 mm for 2002 and 650 mm in 2010 and a daily mean temperature ranging between 15.9 and 39.9 ºC (Tagesson et al. 2015). The raining season is in general relatively short from July to October, with August being the wettest month (Tagesson et al. 2015). The soil is sandy, and the natural vegetation consists mainly of grass and sparse trees such as *Acacia tortilis* subsp. *radiana, A. senegal* and *Balanites aegyptiaca* (Göttsche et al. 2016).

2.1. Experimental design and climatic condition of the site trial

The trial was established from seeds collected from 18 populations (origins, referred to as provenances) across the distribution area of *A. senegal* in Africa and Asia using standard protocols. The design was a randomized complete block design with four blocks. Each block is divided into 18 plots, representing the 18 populations originally with 25 trees each and a spacing of 5 x 5 meters, i.e. 1800 trees at the time of establishment. The survival rate at the time of assessment was 12% with 225 trees and with only 16 populations left of the 18 populations (Table 1). The ploidy level was determined for all the 225 living trees in October 2018 by flow cytometry analysis (Diatta et al. (2021, Manuscript 1)). This present study only concerns 177 diploid and 29 tetraploid trees that were still alive at the end of the assessment in 2019, i.e. 206 trees. Daily observations of rainfall and temperature were available from local climate station (15°24′10″N, 15°25′56″W).

Table 1 Origins and environmental characteristics of *A. senegal* populations used in the study. Provenances are arranged according to WAI0$_{i}$.

Provenance	Country	Latitude	Longitude	Altitude	Mean annual	Mean annual	WAI0$_{i}$	WAI0$_{f}$	#WAI	N0 diploid	N0 tetraploid (4n)
				(masl)	temperature(ºC)	rainfall (mm)	(MOY)	(MOY)	(mm)	(2n) trees	trees
Sodera	Ethiopia	08º24’ N	39º23’ E	1500	21.4	762	4.9	9.0	292	0	5
India60	India	26º19’ N	79º31’ E	120	25.9	867	7.0	8.7	80	0	9
Somo	Mali	13º17’ N	04º54’ W	40	27.5	627	7.1	8.7	187	5	0
Di	Burkina Faso	13º10’ N	03º25’ W	260	28.0	637	7.3	8.2	121	3	0
Bissiga	Burkina Faso	12º26’ N	00º32’ W	280	28.3	696	7.3	8.4	128	5	0
Kidira	Senegal	14º28’ N	12º13’ W	39	28.9	611	7.3	8.9	184	14	1
Sudan	Sudan	12º44’ N	29º35’ E	620	26.8	408	7.4	7.8	56	9	0
Ngane	Senegal	14º08’ N	16º12’ W	2	27.9	570	7.6	8.8	194	7	7
Karofane	Niger	14º18’ N	06º11’ E	280	28.4	366	7.7	7.8	51	14	1
Chad	Chad	12º49’ N	15º18’ E	280	29.0	349	7.8	7.9	64	9	0
Kirane	Mali	15º23’ N	10º15’ W	140	29.3	380	7.8	8.2	59	7	4
Aite	Mali	15º05’ N	11º39’ W	80	29.7	449	7.9	8.2	75	13	2
Kankoussa	Mauritania	15º56’ N	11º27’ W	80	29.7	294	7.9	8.2	60	27	0
Daiba	Senegal	15º22’ N	13º08’ W	28	29.6	437	8.1	8.5	65	13	0
Djigueri	Mauritania	15º44’ N	08º40’ W	226	28.9	309	8.1	8.1	45	26	0
Diamenar	Senegal	16º00’ N	15º54’ W	20	27.1	288	8.4	8.4	28	25	0

#Water availability index calculated as the yearly sum of monthly rainfall minus potential evapotranspiration estimates which are positive and for the period 1961-1990. Estimates of rainfall and evapotranspiration are from the Climate Research Unit, East Anglia University (Harris et al., 2014). WAI0$_{i}$: initial WAI0 defined as the first MOY with WAI > 0; WAI0$_{f}$: final WAI0 defined as the last MOY with WAI > 0. Reference period 1961-1990.

The first day with precipitation in 2018 was registered day 157 (6th June) with 0.05 mm while rain > 2 mm occurred at day 178 (27th June). More continuous rain was registered from day 236 (24th August) in 2018. In 2019, the first day with rain was registered day 181 (30th June) with 0.6 mm while rain > 2 mm occurred at day 205 (24th July), i.e. a month later compared to the previous year. More continuous rain was registered from day 228 (16th August). The last day with rain > 2 mm was day 272 (29th September) and day 298 (25th October) in 2018 and 2019, respectively (Fig. 1). The total annual rainfall recorded in 2018 and 2019 was 295 mm and 355 mm, respectively.

The daily maximum temperature varied between 25 ºC and 44 ºC in 2018, and between 28º and 45º in 2019, with two peaks (inflection) corresponding to days 120 (30rd April) and 300 (27th October) in 2018, and days 140 (20th Mai) and 320 (16th November). The daily minimum temperature varied between 12º and 28º in 2018, and between 14º and 30º in 2019 (Fig. 1d).

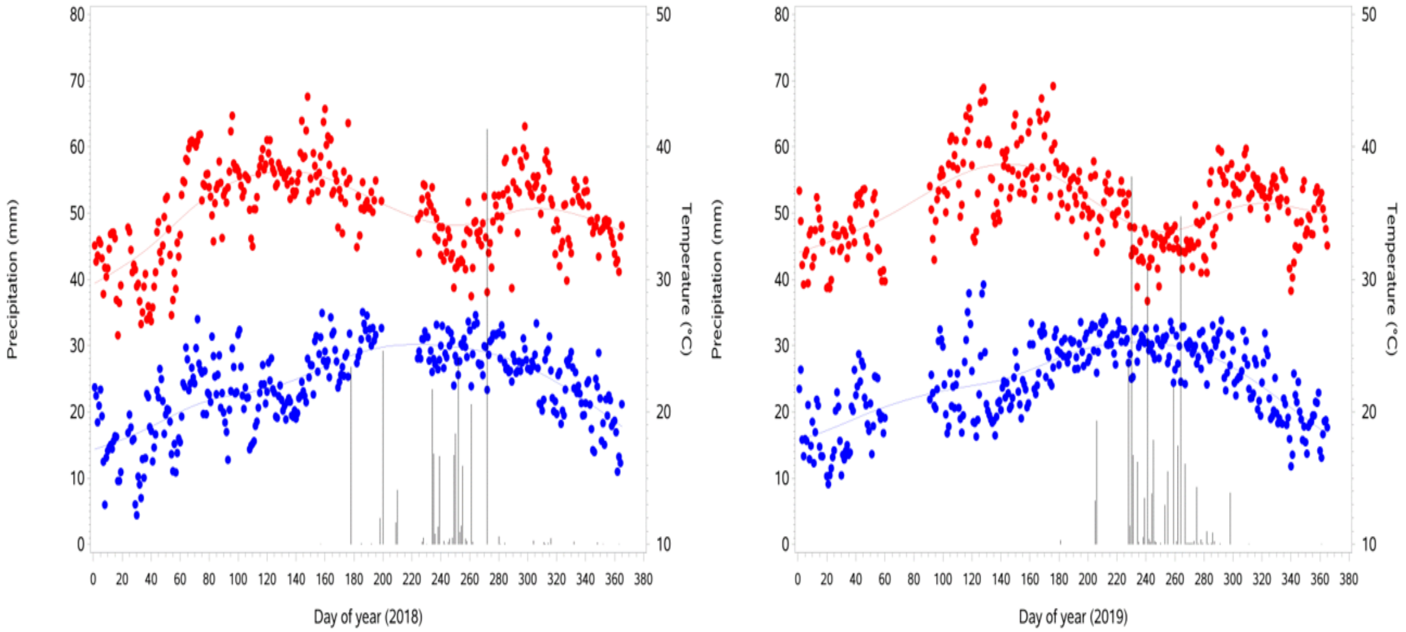

Fig. 1 Daily measures of precipitation (bars), maximum temperature (red dots) and minimum temperatures (blue dots) from the weather station nearest to the Dahra field site (15º40'N, 15º43' W) in 2018 and 2019.

For each site of population origin, monthly water availability indices (WAI) were estimated as the differences between estimates of rainfall and potential evapotranspiration and these were used to estimate a cumulated WAI (WAI0) for the months of each year where WAI was above 0. The estimated WAI0 ranged from 64 mm to 292 mm (Table 1). The estimates of annual precipitation, potential evapotranspiration and mean annual temperatures were from the Climate Research Unit at East Anglia University (Harris et al. 2014) and for the period of 1961-1990. The above estimates were also used to estimate the first month of the year (MOY) with WAI > 0 ($\mathrm{WAI0}_i$), and the last month of the year (MOY) with WAI > 0 ($\mathrm{WAI0}_f$) for the period 1961-1990.

2.2. Phenology assessments

The phenology was assessed every week from August 2nd, 2018 (day 214 of 2018) to December 26th, 2019 (day 360 of 2019). At each assessment, the phenological stages (of leaves, flowers, and fruits) of all trees were recorded (Table 2).

Table 2 Phenological phases for leaves, flowers and fruits as recorded for each tree and their scores.

	Scores	Phases
Leafing	0	No buds
	1	Buds visible
	2	Buds developed
	3	Signs of leaves
	4	Leaflets visible
	5	Leaf expansion
	6	Leaf complete
	7	10-50% brown or shed leaves (senescence i)
	8	>50% brown or shed leaves (senescence ii)
Leaf development (i)	Length from occurrence of "Buds visible" to "Signs of leaves"	
Leaf development (ii)	Length from occurrence of "Signs of leaves" to "Leaf complete"	
Growing period (i)	Length from occurrence of "Buds visible" to "Leaf complete"	
Growing period (ii)	Length from occurrence of "Signs of leaves" to ">50% brown or shed leaves"	
Flowering	0	No flower buds
	1	Flower buds
	2	Fully developed flowers
	3	Withered flowers
Flowering period	Length from occurrence of "Flower buds" to "Withered flowers"	
Fruiting	0	No fruits
	1	Fruit initiation
	2	10-50% fruits developed
	3	>50% fruits developed
	4	10-50% fruits ripe
	5	All fruits ripe

As different parts of the trees could be in different phases of phenology, the dominant phase of the tree was recorded. The day of change from one dominant phase to another was estimated as the day between two assessments with different phenology phases. Two measures for the start of leaf senescence were estimated as respectively (i) day of change from a phase before 7 to phase 7 with "10-50% brown and shed leaves", and (ii) day of change from a phase before 8 to phase 8 with ">50% brown and shed leaves". The growing period (GP) with assumed photosynthetic activity of the leaves of a tree was defined and estimated as respectively (i) the number of days between the first day with registration of "signs of leaves" and the first day with registration of "10-50% brown and shed leaves" (GP (i)), and (ii) the number of days between the first day with registration of "signs of leaves" and first day with registration of ">50% brown and shed leaves (GP (ii))". The leaf development speed was estimated as (i) the number of days between the first registration of "buds visible" and first registration of "leaf complete" (LD (i)), and (ii) the number of days between the first registration of "signs of leaves" and first registration of "leaf complete" (LD (ii)).

The length of flowering period (FP) was estimated for each tree as the number of days between the first day with registration of "flower buds" and the first day with registration of "withered flowers". The frequency of diploid and tetraploid trees that reached "fully developed flowers" was plotted (heatmap) to explore the potential presence of a phenological barrier between cytotypes in *A. senegal.* The two populations, Kirane (Mali) and Ngane (Senegal), that had mixed ploidy levels (diploid and tetraploid individuals growing at the same site), and with a fair number of each cytotype was chosen to test this later hypothesis.

The frequency of trees that developed ripened fruits was calculated by block and population to test for the population differences in years, 2018 and 2019.

2.3. Statistical analysis

The phenological development of leaves, flowers and fruits for diploid and tetraploid populations were analyzed using the R statistical software (R Core Team 2020). The R package *ggplot2* (Wickham 2016) was used to illustrate phenological patterns.

As the frequency of polyploid individuals differed highly among populations, effects of geographic origin and ploidy level were confounded. We therefore used a two steps approach for the analyses. First, we analyzed the effect of ploidy for only the five populations where both diploid and tetraploid trees were present, based on the general linear model (model 1):

$$\mathbf{Y_{ijk} = \mu + B_i + G_j + P_k + \varepsilon_{ijk},} \qquad \textbf{(1)}$$

where Y_{ijk} is the mean estimated day for a change in phenology phase by block and population, μ is the general mean, B_i is the random effect of block i, G_j is the fixed effect of geographic origin j, P_k is the fixed effect of ploidy level k and ε_{ijk} represent the residual errors assumed to be independent and normal distributed $(0, \sigma^2)$. This model was applied to test the effect of ploidy.

The second step was to analyze the variation among origins. Here, we made the analysis separately for diploid and tetraploid trees based on the following model to avoid confounding effects of origin and ploidy (model 2):

$$\mathbf{Y_{ij} = \mu + B_i + G_j + \varepsilon_{ij},} \qquad \textbf{(2)}$$

with abbreviation of effects as above. Here, we thus made two analyses for each trait, one for the diploid trees and one for the tetraploid trees.

The function *lmer* (Linear Mixed-Effect Models) in the package *lme4* (Bates et al. 2015) for R (R Core Team 2020) was applied and using the Kenward-Roger adjusted degrees of freedom. Residuals were plotted against predicted values to examine for heteroscedasticity and outliers and histogram of residuals were examined for normality.

Pearson's correlation coefficients were estimated between population means (least square mean estimates) for flushing, flowering phenology and growth (height, diameter, and crown area measured in year 2017). To estimate the population means in growth, model (2) was applied respectively for the diploid and tetraploid populations and the derived least square means were used to test the correlations with phenology. In addition, phenology was correlated to climate variables at the site of origin of populations, and significant correlations visualized by plots. The correlation coefficients and associated tests of significance based on the population least square mean estimates were carried out for diploids and tetraploids separately using the *corr.test* function in the package *psych* (Revelle 2019) as implemented in R (R Core Team 2020).

3. Results

3.1. Leafing, flowering and fruiting phenology in *A. senegal* with relation to environmental conditions

In 2019, leaf buds started to appear on a few trees around day 94 (first week of April) (Fig. 2b). Buds developed progressively before the first day with rain > 2 mm (day 205), while “leaf complete” was registered from day 220 (cf. Fig. 2a, b). All trees had complete leaves at day 241 (Fig. 2b). The first trees showed signs of senescence (i) at day 270 in 2018 (the last week of September), and day 276 in 2019 (first week of October). This was slightly earlier than the end of the rainy season for both years (cf. Fig. 2a, b).

In general, flowers and fruits were produced during the periods of more continuous rainfall (Fig. 2c, d). In 2019, a low frequency of trees (1.5%) produced flowers at day 66 and 213 before the more continuous rainfall, but all these flowers were aborted (Fig. 2c). Flowering took place within 15 weeks (days 214 - 312) in 2018, and 12 weeks (day 241 - 318) in 2019 (Fig. 2c). Fruit initiation was observed three and two weeks after the observation of the first flower buds in 2018 (day 236) and 2019 (day 255), respectively. Fruits began to ripen towards the end of the rainy season at day 284 (11th October 2018) and day 304 (31st October 2019) (Fig. 2d). The frequency of trees that completed all fruiting phases was 97% in 2018 while only 32% of the surviving trees set fruits in 2019. These differences suggest a higher frequency of fruit abortion in 2019 (Fig. 2d), which may be due to the fact that trees initiated fruit development almost three weeks later in 2019 (day 255) compared to 2018 (day 249).

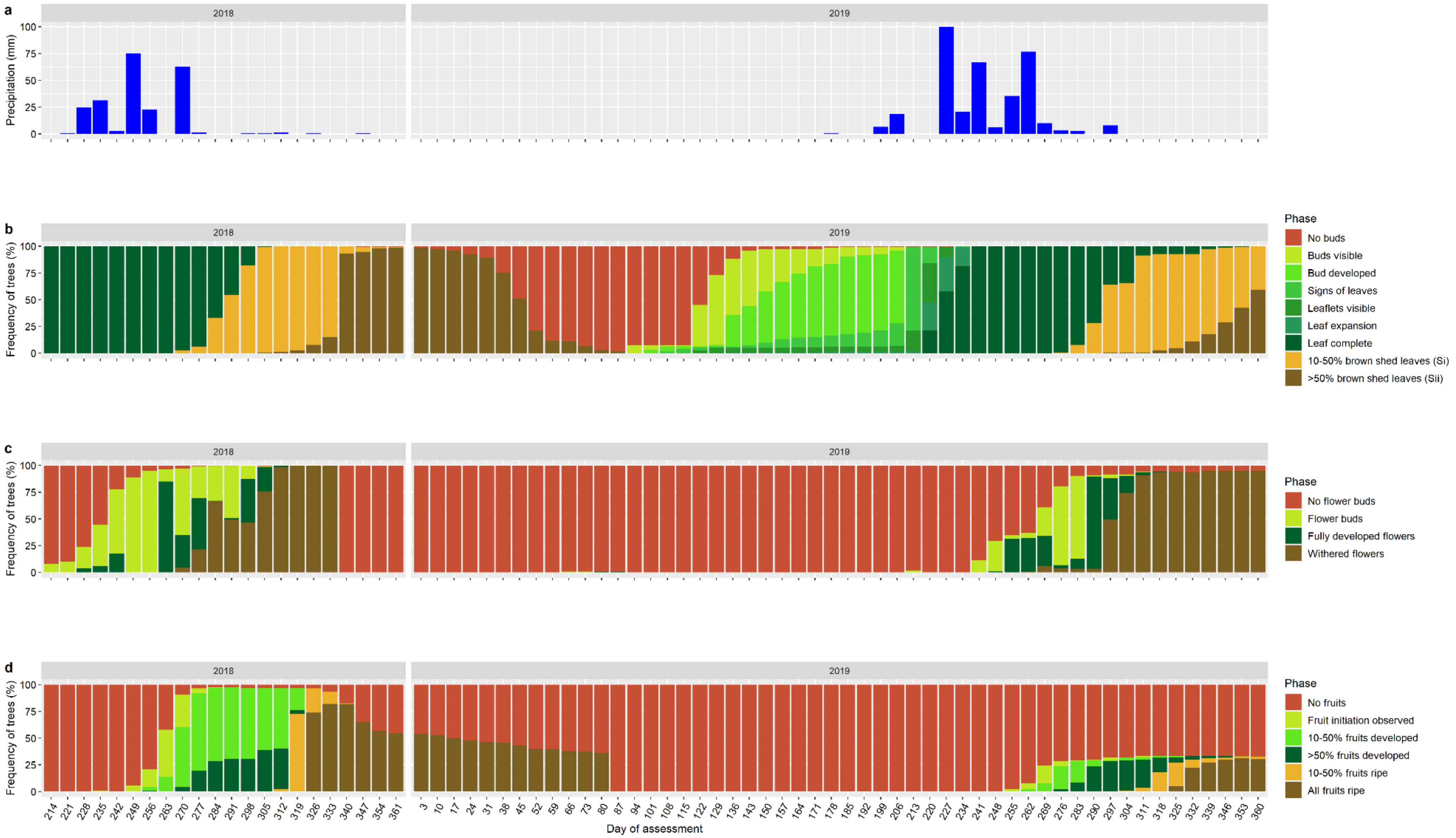

Fig. 2 Patterns of rainfall (a), leafing (b), flowering (c) and fruiting (d) phenology of *A. senegal* during the period of survey (August 2018-December 2019). Weekly accumulate rainfall (blue bars) from the weather station nearest to the Dahra field site (15°24′10″N, 15°25′56″W) in 2018 and 2019.

3.2. Variation in leafing phenology between ploidy levels and among populations

Diploids and tetraploids differed significantly in leafing phenology as regards the day with “signs of leaves”, the number of days to “leaf complete” (LD(ii)), and the length of the growing period (GP(i)). Tetraploids thus showed earlier “signs of leaves” (day 187± 7.3) and longer length of LD (ii) (day 43 ± 7.3) as compared to diploids ones (day 203 ± 5.2 and day 26 ± 5.1, respectively) (Table 3). Since diploids and tetraploids reached “leaf complete” almost at same time, the diploid trees had a significantly faster LD compared to tetraploids trees (Table 3).

Diploid populations were significantly different from each other in regards to the first day of leaf senescence (i) in 2018, but this result was not repeated in 2019. In addition, diploid populations differed in first day with “buds visible”, “buds developed”, “signs of leaves”, “leaf expansion” and “leaf complete” (Table 3). The four populations Daiba, Diamenar, Ngane (Senegal) and Djigueri (Mauritania) had the greater frequency of trees (≥ 25%) starting early leaf flushing (first day with “buds visible” and first day with “buds developed”) among the diploid populations (Fig. S1). These populations originate from dry sites where a positive monthly WAI0 occurs late in the year (Table 1),

Tetraploid populations showed differences from each other in senescence in both 2018 and 2019 at least concerning the first day with “10-50% brown or shed leaves” (senescence (i) 2018 and 2019; Table 3). Significant differences were also found in first day with “buds developed”, “leaflets visible”, and “leaf complete” (Table 3). The local population Ngane had early leaf flushing (first day with “buds visible” and first day with “buds developed”) among tetraploid populations (Fig. S2).

The population differences in leaf flushing and senescence were also to some extent reflected in significant differences, among diploid populations in LD (i) and GP (i), and among tetraploid populations in both estimates of GP (Table 3).

Table 3 Variation in leafing phenology between diploid and tetraploid trees of *A. senegal* based on data from populations represented by diploid and tetraploid trees (*model 1)* and among diploid and tetraploid populations (*model 2*). Variables where no year is specified were recorded in 2019.

Variables	Mean values				Statistical results								
					Ploidy levels (1)			Population tests (2)					
								Diploids			Tetraploids		
	Diploid	n	Tetraploid	n	Df	F value	P value	Df	F value	P value	Df	F value	P value
Senescence (i) (2018)	291 ± 1.5	55	291 ± 2.5	15	1	0.00	0.9751	13	4.13	**<0001*** **	6	10.13	**<0001*** **
Senescence (ii) (2018)	338.8 ± 0.8	55	335.8 ± 1.4	15	1	3.43	0.069	13	2.10	**0.0154***	6	1.91	0.1311
Buds visible	127 ± 2.8	55	125 ± 4.0	15	1	0.39	0.537	13	2.80	**0.0012****	6	2.37	0.0707
Buds developed	154 ± 3.3	55	151 ± 6.2	15	1	0.16	0.6912	13	2.50	**0.0041****	6	6.65	**0.0008****
Leaf signs	203 ± 5.2	55	187 ± 7.3	15	1	5.98	**0.0180***	13	2.18	**0.0127***	6	2.48	0.0685
Leaflets visible	214 ± 5.6	55	203 ± 7.3	15	1	2.28	0.1408	13	1.71	0.0718	6	3.28	**0.0314***
Leaf expansion	224 ± 5.7	55	226 ± 1.8	15	1	1.3	0.2624	13	3.18	**0.0006****	6	0.34	0.8886
Leaf complete	229 ± 1.1	55	230 ± 1.8	15	1	0.21	0.6504	13	3.54	**0.0001*** **	6	9.07	**0.000*** **
Senescence (i) (2019)	301 ± 2.1	55	303 ± 4.2	15	1	0.15	0.6986	13	0.87	0.5867	6	3.45	**0.0177***
Senescence (ii) (2019)	349 ± 2.9	55	346 ± 4.6	15	1	0.22	0.6426	13	0.77	0.6902	6	1.99	0.1947
Leaf development (i)	101 ± 2.8	55	105 ± 4.4	15	1	0.59	0.4439	13	1.90	**0.0335***	6	1.34	0.2878
Leaf development (ii)	26 ± 5.1	55	43 ± 7.3	15	1	6.55	**0.0136***	13	1.64	0.0796	6	2.21	0.096
Growing period (i)	98 ± 5.7	55	114 ± 8.1	15	1	3.58	**0.064**	13	0.08	**0.0087***	6	7.52	**0.0003*** **
Growing period (ii)	145 ± 6.03	55	164 ± 8.9	15	1	3.85	0.058	13	0.851	0.6064	6	21.59	**0.0003*** **

Least square means ± SE of the distribution and standard error, Df: degrees of freedom. Asterisks indicate the level of significance; *p<0.05; **p<0.01; ***p<0.001

3.3. Relationship between leaf flushing and growth traits

In both diploid and tetraploid populations first day with “signs of leaves” and “leaflets visible” correlated negatively with the number of days for LD (ii) indicating that late flushing populations in 2019 reached “leaf complete” about the same time as the populations with early leaf flushing. Nevertheless, both estimates of GP (GP(i) and GP(ii)) were negatively correlated with first days of the early flushing phases, particularly first day with “signs of leaves” and “leaflets visible”, which means that the early flushing trees in general had a longer GP (Table S1).

In diploid populations, the first day of “buds developed” (2019) was negatively correlated with height and crown area (Fig. 3a,c). The senescence (i) in 2019 and GP (i) correlated positively with the diameter (Table S1, Fig. 3h). Thus, populations with early “buds developed” developed taller trees and larger crown areas compared to populations with late “buds developed”, and populations with late senescence had a longer GP and larger diameter.

In tetraploid populations, the two estimates for the start of senescence (senescence (i) and senescence (ii) were in 2018 positively correlated with growth (Table S1, Fig. 3d,e,f). The significant correlations were mainly due to the two populations India60 (India) and Sodera (Ethiopia), and no clear relationships were found when these two populations were removed from the analysis (Fig. S3d,ef).

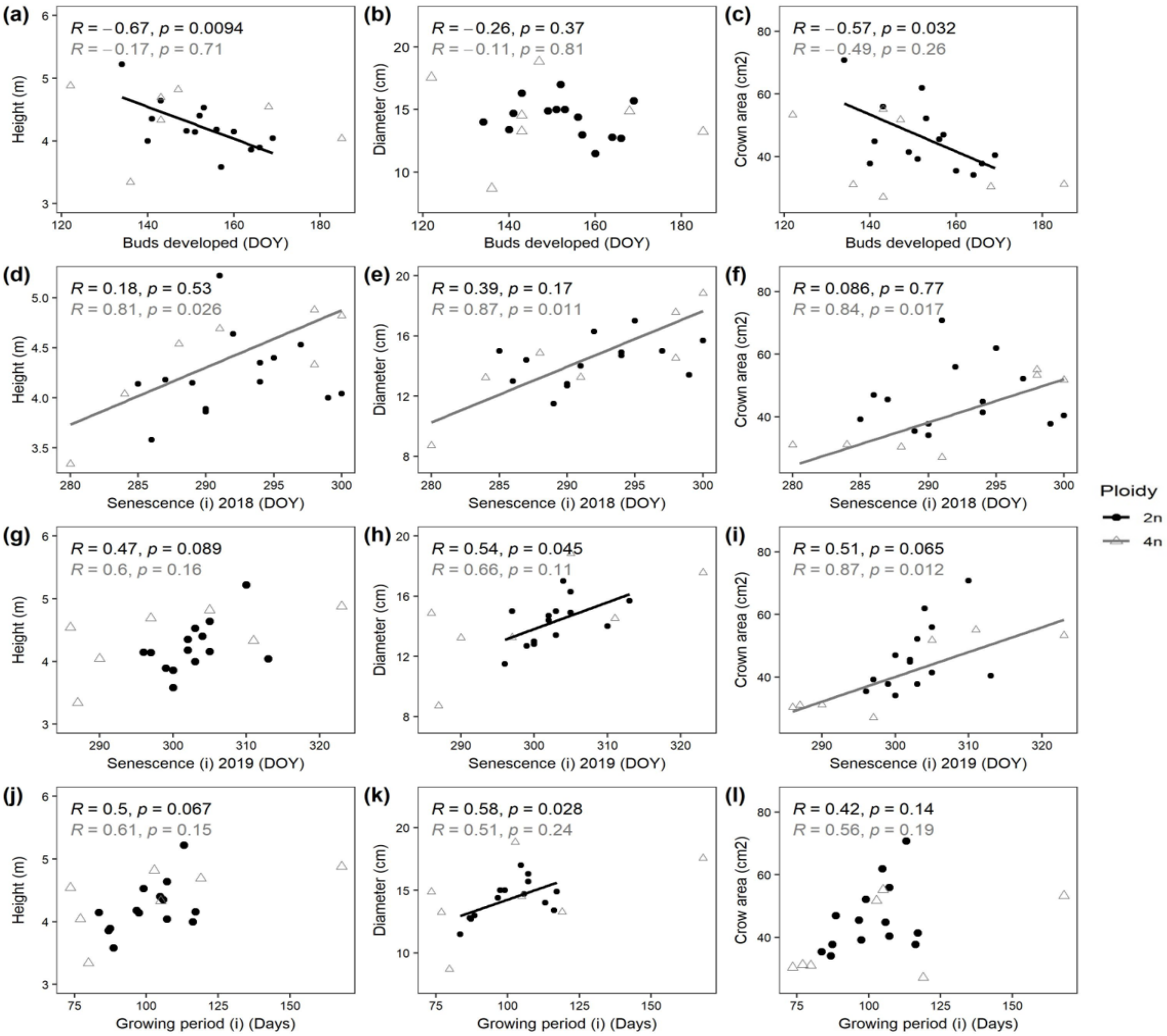

Fig. 3 Pairwise plots of the growth variables (height, diameter and crown area) against estimates of first day with developed buds (a, b, c), first day with senescence (i) in 2018 (d, e, f) and 2019 (g, h, i) and growing period (GP (i)) (j, k, l). Symbols represent population means. Dark symbols denote diploid populations, whereas grey symbols denote tetraploids. DOY means day of the year. The correlations in Fig. 3d, e, f are not significant (p = 0,31, p = 0,23, p = 0,057, respectively) when India60 and Sodera were removed.

3.4. Relationship between leaf flushing and climate at the site of origin

Among diploid populations, the timing of “signs of leaves” and “leaflets visible” was earlier among populations from sites where the first month of the year with WAI > 0 ($WAI0_i$) occurred late (Fig. 4a, b) i.e., typically dry sites. The speed of LD (ii) and GP (ii) was correspondingly longer for populations from sites where a positive monthly $WAI0_i$ appeared late (Fig. 4c, d). In tetraploid populations, there were no clear relationship between leafing phases and the climate variables.

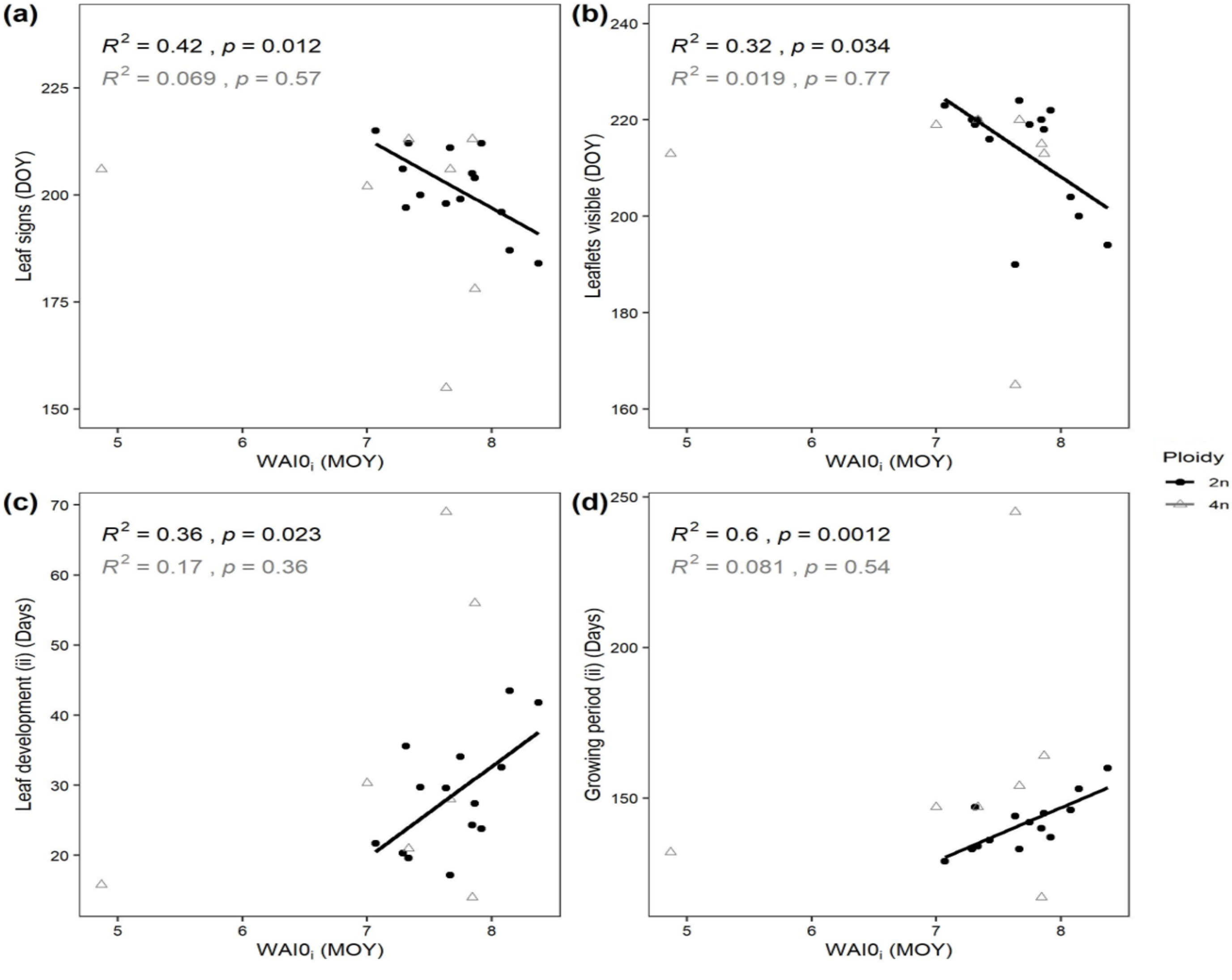

Fig. 4 Pairwise plots of the leaf signs, leaflets visible, leaf development (ii) and growing period (ii) against the $WAI0_i$ (first month of year (MOY) with WAI > 0 average for the period 1961-1990) (a,b,c,d), in diploid and tetraploid populations. Symbols represent population least square means. Growing period (ii) is the number of days between the first day with registration of “signs of leaves” and first day with registration of “>50% brown and shed leaves” (GP (ii)); The leaf development (ii) the number of days between the first registration of “signs of leaves” and first registration of “leaf complete” (LD(ii)).

3.5. Variation in flowering phases between ploidy level and among populations

The time point where diploids and tetraploids had a dominance of “fully developed flowers” and “flowers withered” differed significantly in 2019 (for tetraploids: “fully developed flowers” at day 269 ± 4.7 and “flower withered” at day 291 ± 3.1 as compared to diploids at day = 279 ± 2.8 and 299 ± 1.9, respectively) (Table 4). No significant differences between ploidy levels were found for flowering phases in 2018.

In diploid populations, significant differences were found in first day with “withered flowers” in 2018 and 2019, and first day with dominance of “fully developed flowers” in 2019.

Among tetraploids populations, significant differences were found in the initiation of flower buds and the length of flowering period (FP) in 2019 (Table 4).

Table 4 Variation in flowering phases between diploid and tetraploid trees of *A. senegal* based on data from populations represented by diploid and tetraploid trees (*model 1*) and among diploid and tetraploid populations (*model 2*).

Variables	Mean values				Statistical results								
					Ploidy levels (1)			Population tests (2)					
								Diploids			Tetraploids		
	Diploid	n	Tetraploid	n	Df	F value	P value	Df	F value	P value	Df	F value	P value
Flower buds (2018)	237 ± 2.2	55	238 ± 3.7	15	1	0.07	0.789	13	0.58	0.8677	6	0.58	0.7391
Fully developed flowers (2018)	257 ± 2.7	55	260 ± 4.0	15	1	0.45	0.505	13	0.49	0.9293	6	0.68	0.6704
Flowers withered (2019)	287 ± 1.5	55	284 ± 3.0	15	1	0.73	0.398	13	2.24	0.0101*	6	1.29	0.3086
Flowering length 2018	51 ± 2.4	55	45 ± 4.6	15	1	1.47	0.231	13	1.74	0.0571	6	0.61	0.7194
Flower buds (2019)	264 ± 2.8	55	254 ± 5.1	15	1	2.87	0.096	13	1.08	0.3855	6	7.49	0.0006**
Fully developed flowers (2018)	279 ± 2.8	55	269 ± 4.7	15	1	4.15	0.0466*	13	1.95	0.0298*	6	1.27	0.3182
Flowers withered (2019)	299 ± 1.9	55	291 ± 3.1	15	1	7.09	0.0104*	13	3.83	<0001***	6	0.79	0.5896
Flowering length 2019	35 ± 2.3	55	36 ± 4.5	15	1	0.04	0.8488	13	0.91	0.5439	6	43.53	<0001***

Least square means ± SE of the distribution and standard error, Df: degrees of freedom. Asterisks indicate the level of significance; $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

The similar time point for a high frequency of trees that reached “fully developed flowers” within the populations Kirane and Ngane suggested a period of overlap in flowering time between diploid and tetraploid trees (Fig. 5).

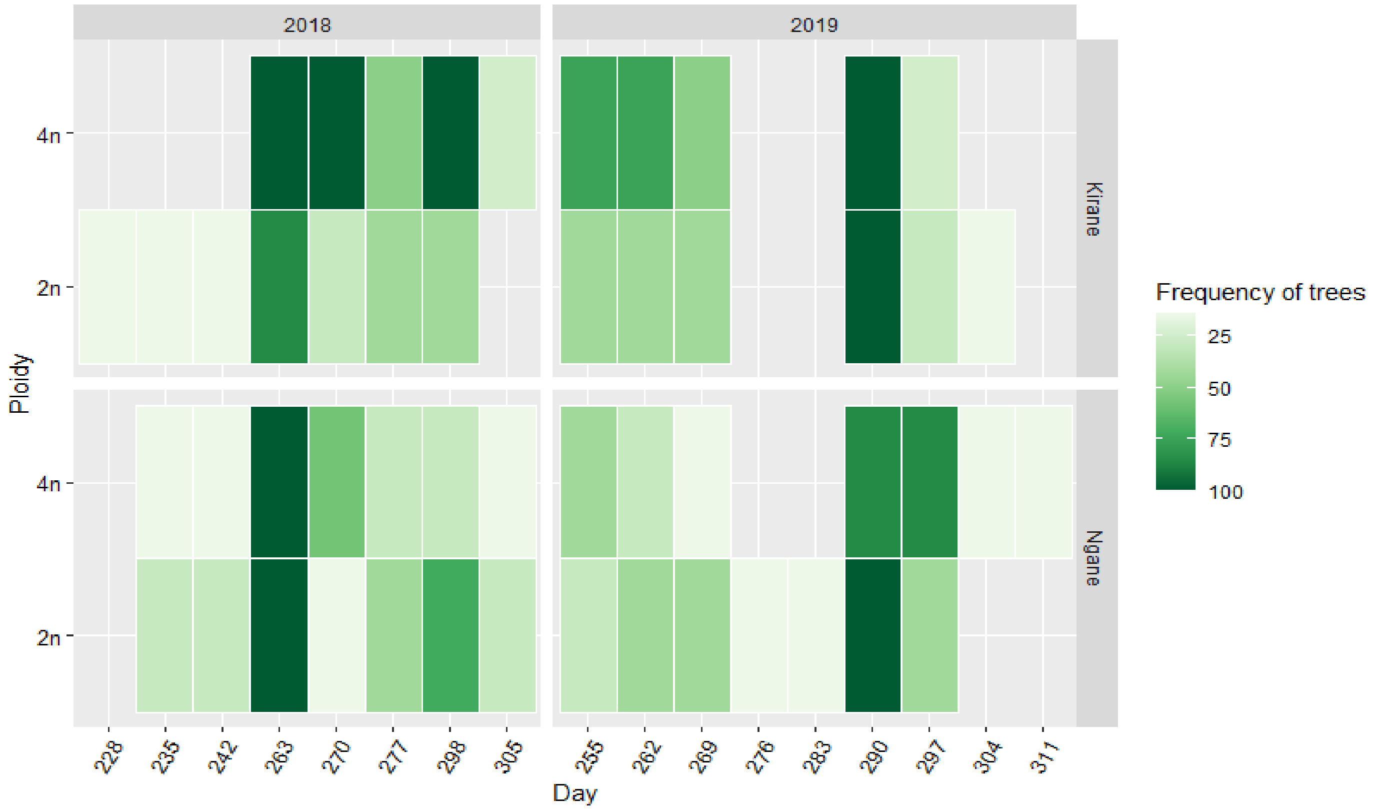

Fig. 5. Frequency of fully developed and likely fertile flowers among diploid and tetraploid groups of trees within the two populations Kirane and Ngane, respectively.

3.6. Variation in fruiting phases between ploidy level and among populations

Significant differences were found between diploids and tetraploids in the early fruiting stages in 2018 ("fruit initiation" and "10-50% developed fruits" with diploids having early fruit initiation and development (diploids: 263 ± 0.8 and 271 ± 1.4, respectively and tetraploids: 270 ± 1.5 and 276 ± 1.8, respectively) (Table 5). Variation among diploid populations was significant in the timing of almost all fruiting phases, except for ">50% fruits developed" and "all fruits ripe" in 2018 and 2019 respectively (Table 5). In tetraploid populations, populations were only significantly different in the timing of their early fruiting phases ("fruit initiation" and "10-50% developed fruits") and fruit ripening ("10-50% fruits ripe" and "all fruits ripe") in 2018 and 2019 respectively. However, a low number of tetraploid trees had developed ripe fruits in 2019, which explained the higher significance among populations.

Table 5 Variation in fruiting phases between diploid and tetraploid trees of *A. senegal* based on data from populations represented by diploid and tetraploid trees (*model 1*) and among diploid and tetraploid populations (*model 2*)

Variables	Mean values				Statistical results								
					Ploidy levels (1)			Population tests (2)					
								Diploids			Tetraploids		
	Diploid	n	Tetraploid	n	Df	F value	P value	Df	F value	P value	Df	F value	P value
Fruit initiation 2018	263 ± 0.8	55	270 ± 1.5	15	1	19.30	< 0001***	13	2.36	0.0069**	6	16.44	<0001***
10-50% fruits developed 2018	271 ± 1.4	55	276 ± 1.8	15	1	13.36	0.0005***	13	2.93	0.0008***	6	6.69	0.0006***
>50% fruits developed 2018	286 ± 2.4	55	287 ± 5.5	15	1	0.02	0.8967	13	1.18	0.3147	4	2.51	0.3046
10-50% fruits ripe 2018	320 ± 0.5	55	320 ± 0.6	15	1	1.74	0.1930	13	5.4	< 0001***	6	1.7	0.1753
All fruits ripe 2018	328 ± 0.60	55	328 ± 0.87	15	1	0.10	0.7476	13	6.9	< 0001***	6	1.01	0.4492
Fruit initiation 2019	269 ± 3.6	55	270 ± 4.0	15	1	0.29	0.6060	11	2.63	0.012*	4	2.69	0.1803
10-50% fruits developed 2019	278 ± 5.3	55	280 ± 5.8	15	1	0.32	0.5840	11	3.96	0.0006***	4	1.103	0.4348
>50% fruits developed 2019	289 ± 3.1	55	290 ± 3.7	15	1	0.33	0.5846	11	2.79	0.0081**	4	0.033	0.9968
10-50% fruits ripe 2019	318 ± 2.2	55	322 ± 3.4	15	1	2.28	0.1621	11	2.23	0.0307*	4	2.33E+28	<0001***
All fruits ripe 2019	332 ± 1.3	55	338 ± 3.0	15	1	3.13	0.1072	11	1.13	0.3682	4	2.33E+27	<0001***
Mean % trees making fruits 2018	97 ± 1.8	55	100 ± 2.3	15	1	1.5	0.2314	13	1.26	0.2838	6	1.00E+00	0.4326
Mean % trees making fruits 2019	34 ± 13.5	55	29 ± 16.0	15	1	0.11	0.7419	13	1.35	0.2341	6	20.31112	0.0023

Least square means ± SE of the distribution and standard error, Df: degrees of freedom. Asterisks indicate the level of significance; *p<0.05; **p<0.01; ***p<0.001.

No significant differences were found between diploid and tetraploid trees, and among diploid populations in their success of developing ripened fruits in 2018 and 2019 (Table 5).

4. Discussion

The present study illustrated variation in phenological events of *A. senegal* when grown in the African dryland (Senegal), and revealed for the first time the adaptive potential of different origins and ploidy levels to the prevailing climate.

4.1. Triggering factors in relation to phenological patterns

In this present study, *Acacia senegal* shed most of its leaves during the dry season and leaf development began before the rainy season. The full development of the leaf, flowering and fruiting phases occurred during the rainy season. Our finding of leafing initiation before rainfall is in line with another study on phenology of *A. senegal* in West Africa (Seghieri et al. 2012a) and with the general findings of pre-rain green up in the dry tropics from satellite images (Ryan et al. 2017). The findings are however in contrast to a previous study on phenology of *A. senegal* in Kenya (Omondi et al. 2016), where the initiation of leaves were found to occur at the onset of the rain. In contrast to our study the phenology of Kenyan *A. senegal* was bimodal and followed the rainfall patterns. In the dry tropics, the phenomenon of leafing before the rainy season may be triggered by other climatic factors such as photoperiodicity, temperature and air humidity (De Bie et al. 1998; Myers et al. 1998; Do et al. 2005; Seghieri al. 2012b; Di Lucchio et al. 2018) . When investigating the phenology of *A. tortilis* subsp. *raddiana* in the Sahelian zone of Senegal, Do et al. (2005) suggested that the relative humidity of the air in connection with low pressures in the Inter-Tropical Convergence Zone (ITZC) is a determinant factor of early leaf flushing in this region, while Segheieri et al. (2012b) asserted that temperature and day-length are the main determining factors for both *A. senegal* and *A. raddiana*. In a greenhouse experiment, Di Lucchio et al. (2018) found that flushing in *A. digitata* was influenced by the day-length with the lowest degree of meristematic activity observed in the short day-length treatment. The environmental factors that influence our observed leafing in *A. senegal* are still not clear. Thus, further investigations under controlled conditions highlighting effects of environmental factors (temperature, day-length, drought) and leafing phenology will be needed to clarify the factors of importance for the leaf phenology of *A. senegal*.

The completion of leaf development as well as the development of flowers and fruits during the rainy season attests to the importance of a match between phenology and local seasonality, in relation to changing conditions in soil water availability (Borchert 1994; Okullo et al. 2004; Omondi et al. 2016). Tree species from dry tropical areas mostly use the favorable but often very short rainy seasons for leaf development to accumulate sufficient photosynthates and initiate reproduction before the soil moisture starts to fall in the subsequent drier season (Singh and Kushwaha 2006). The results also sustained that rainfall usually act as a cue that trigger of flowering (and fruiting) in *A. senegal* (Tandon et al. 2001; Omondi et al. 2016; but see also Tybirk 1992), and allows fruit growth and maturation (Lieberman, 1982).

While climate variables are potential factors that trigger leaf flushing in tropical drylands, the ability to access deep soil moisture (Borchert 1994) or groundwater (Do et al. 2005; Do et al. 2008), and the storage of water in tree stems (Borchert 1994; Myers et al. 1998;) may also be important cues to phenological behavior. In deep-rooting woody plants, the strategy of leafing before the start of the rainy season indicates other available water resources that also provide a fully operating photosynthetic apparatus under favorable conditions (De Bie et al. 1998). In addition, seasonal changes in trees water status with reduction of water loss during leaf shedding in combination with available soil water enabled rehydration of stem tissues and subsequent flowering or flushing during the dry season (Borchert 1994). Leaf flushing during the dry season has been found in *Adansonia* species in Madagascar (Chapotin et al. 2005), and the authors related the early development of leaves to the stem water reserves.

4.2. Genetic variation among populations

In the present paper we found significant genetic variation in leafing phenology among both diploid and tetraploid populations while grown at the same site in Senegal. In general, populations of *A. senegal* with early leaf flushing had a faster leaf development and hence a longer growing period, and it could be speculated if they have resistance to water loss to sustain the period from leaf flushing to start of the rainy season. In addition, the early flushing trees performed better in growth, i.e. the trees were taller and had large crown areas. This suggests an important strategy to benefit from early rainfall and to make leaves ready to start photosynthesizing when the rainy season starts, and too late leaf flushing or too early senescence can be affiliated with the reduction of fitness in *A. senegal*.

The genetic differences in leafing phenology suggest the presence of local adaptation to seasonality in *A. senegal*. Indeed, diploid populations with a late $WAI0_i$, i.e. typically from dry sites, had long-lived leaves and therefore also a longer growing period. Although no significant relationships were found between leafing phases and other climate variables at the origins the $WAI0_i$ was negatively correlated with the annual mean precipitation and also positively correlated with the latitude of the origins. These results support the finding of Diatta et al. (Manuscript 1, in review) on local adaptation to dry conditions, where *A. senegal* provenances from dry sites performed relatively better in height and diameter at the dry site Dahra compared to provenances from wet sites that performed poorly at the dry site. These particular patterns therefore support additional investigations on factors triggering leafing in *A. senegal* and their associated physiological mechanisms.

We do not find any relationships between timing of senescence and climate. This contrasts previous studies of African tropical species such as *A. digitata* (Di Lucchio et al. 2018; Bamba et al. 2019) and *P. biglobosa* (Ouedrago, 2014). These studies of *A. digitata* demonstrated that the timing of senescence was related to the climatic parameters at the site of origin, and the senescence of *P biglobosa* populations followed a latitudinal gradient. In order to enhance knowledge on the adaptation of *A. senegal* to different climatic conditions, more investigations among successive years and in different geographic locations will therefore be needed.

4.3. Genetic variation between ploidy levels

Although it is generally accepted that *A. senegal* is a heteroploid species with different levels of ploidy and that different cytotypes can either co-exist in the same populations or be found in pure populations (Assoumane et al. 2013; Odee et al. 2015; Diallo et al. 2015) it is still not clear how well the cytotypes are delimited and what mechanisms reinforces the potential boundaries. In the present study we found differences between diploids and tetraploids in some leaf phenological characters (the first signs of leaves and the speed of development of leaves). Although tetraploids started to flush earlier (sign of leaves at day 187± 7.3 compared to the diploids with 203 ± 5.2) diploids had a significantly faster leaf development. As regards flowering and fruiting, tetraploids had early fully developed flowers and flower senescence, but the differences in fruit development between the two cytotypes were not clear. The phenological differences between cytotypes suggest that ploidy level can influence the fitness of the trees in *A. senegal*. Since the leaf phenological phases “sign of leaves” and “complete leaves” appeared respectively before and after the raining season, and diploids had faster leaf development despite their late leaf initiation, we can hypothesize that diploid and tetraploid individuals employ different water-use strategies to adapt to contrasting environments. An explanation could be a better performance of *A. senegal* polyploids under drought stress (Diallo et al. 2016), perhaps because tetraploids display higher water use efficiency (WUE_i) compared to diploid individuals (Diatta et al (2021, Manuscript 1)). It has been demonstrated that under conditions of changing water availability, deciduous species such as *A. senegal* do not maintain a consistent water use strategy (Gebrekirstos et al., 2011), and plants displaying higher or lower WUE_i under drought conditions, may respectively exhibit lower or higher WUE_i in wet conditions

(Gebrekirstos et al. 2011; Gray et al. 2013). Further investigations in both dry and growing seasons will be determinant to clarify the degree of fitness between diploid and polyploids in their water use strategies.

Diploid and tetraploids differed in their flowering in 2019, with tetraploid individuals developing fully flowers earlier and with shorter flowering time compared to the diploid ones. However, the same patterns were not observed in 2018 where both ploidy levels seemed to flower simultaneously. This suggests a possibility of pollen transfer within populations despite of ploidy level, and that subspecies are not likely to become established because of differences in timing of peak flowering.

Our study also showed that flowering time did not differ as regards the ploidy levels within the same populations, suggesting a period of overlap between diploid and tetraploid individuals. This lack of phenological separation imply that flowering phenology in *A. senegal* does not pose a strong reproductive barrier between cytotypes that coexist.

5. Conclusion

This study is to our knowledge the first to investigate the variation in phenology among different origins of *A. senegal*. It showed that phenology in *A. senegal* is under both genetic control and environmental influences. Still, the environmental cue that triggers leafing phenology is not yet known and needs further investigation. The results revealed local adaptation to seasonality, with early budburst, long growing season and better performance as characteristics of populations from dry areas. To face the ongoing climatic changes, further research on physiological mechanisms in relation to the leaf life-span in *A. senegal* will be needed for future use in conservation programs in Africa. Our study also suggests further investigations on potential reproductive barriers between diploids and tetraploids of *A. senegal* to shed light on how different cytotypes are maintained.

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Supplementary materials

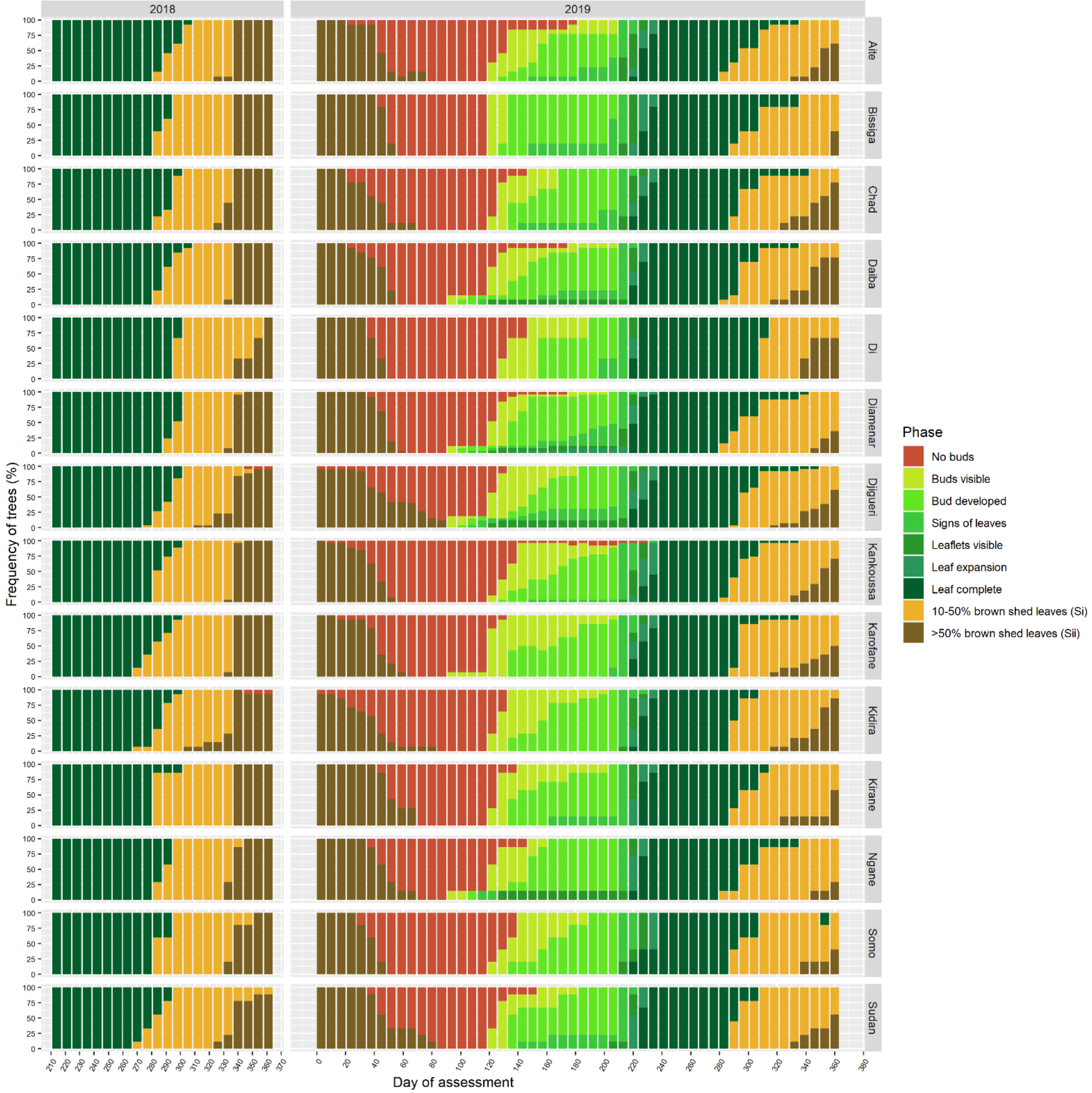

Fig. S1 Variation in leaf phenology among diploid (2n) populations

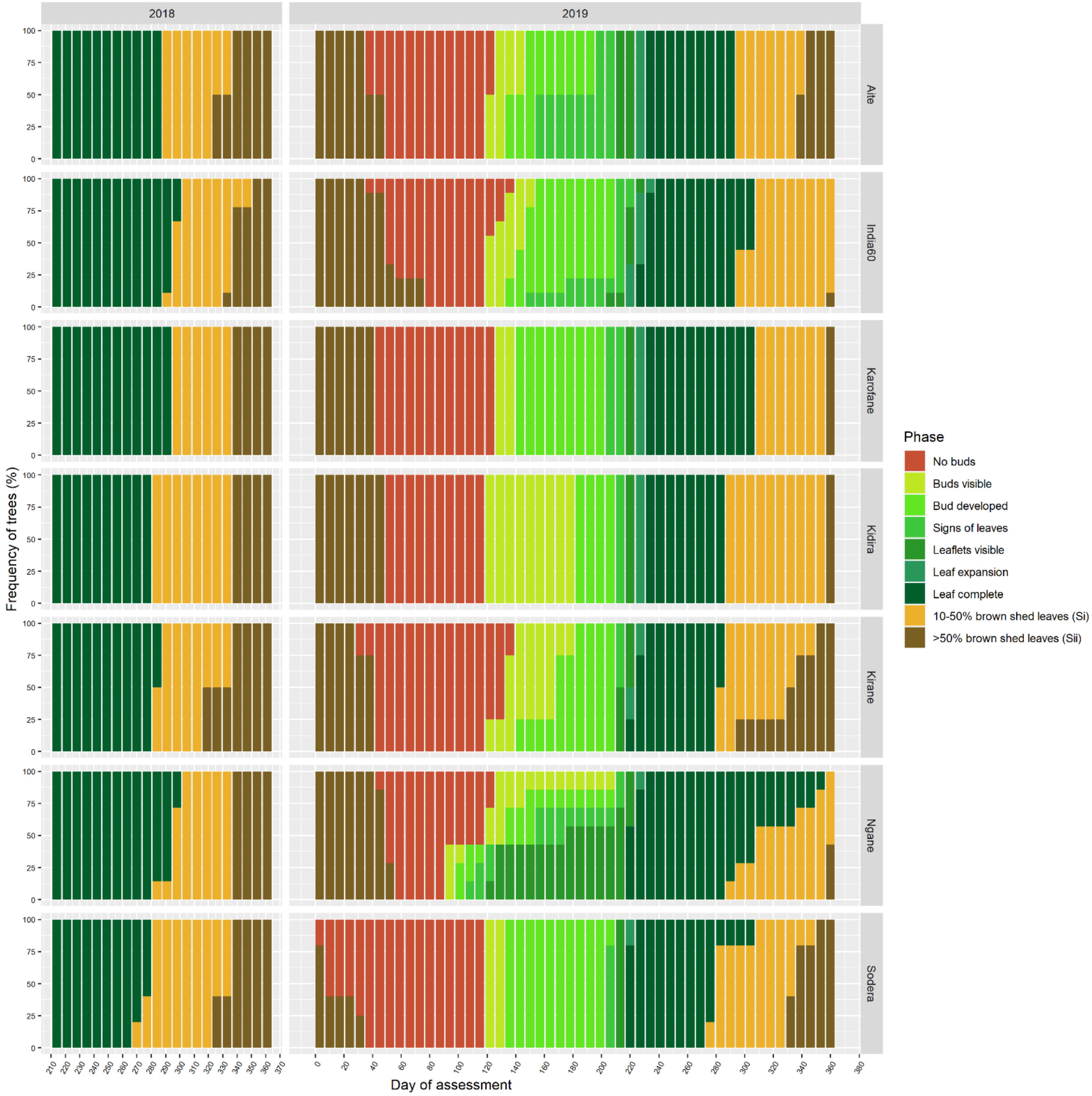

Fig. S2 Variation in leaf phenology among tetraploid (4n) populations

Table S1. Pairwise correlations between leafing phases and growth variables in diploid (upper triangle) and tetraploid (lower triangle) population means

	Buds visible	Bud developed	Leaf sign	Leaflets visible	Leaf expansion	Fully leaves	10-50% shed leaves (2018)	10-50% shed leaves (2019)	<50% shed leaves (2018)	<50% shed leaves (2019)	LD (i)	LD (ii)	GP (i)	GP (ii)	Height	Diameter	Cr
Buds visible	-	**0.71****	**0.56***	0.52	0.25	0.26	0.21	-0.06	0.4	-0.23	**-0.87*****	-0.51	-0.45	-0.51	-0.21	0.02	-0.3
Bud developed	0.47	-	**0.76****	**0.63***	0.28	0.28	-0.11	-0.21	0.38	-0.52	**-0.57***	**-0.74****	**-0.68****	**-0.76****	**-0.67****	-0.26	**-0.57***
Leaf sign	0.71	0.72	-	**0.78*****	0.5	0.49	-0.46	-0.35	0.13	-0.26	-0.28	**-0.94*****	**-0.91*****	**-0.92*****	-0.37	-0.43	-0.25
Leaflets visible	**0.8***	0.62	**0.87***	-	0.46	**0.55***	-0.38	-0.17	0.13	-0.21	-0.24	**-0.68****	**-0.67****	**-0.74****	-0.22	-0.32	-0.03
Leaf expansion	-0.14	0.12	-0.38	-0.2	-	**0.86*****	-0.22	-0.34	-0.3	0.07	0.16	-0.2	-0.51	-0.35	-0.09	-0.35	-0.03
Fully leaves	0.4	0.46	0.22	0.52	0.72	-	-0.4	-0.4	-0.4	0.23	0.24	-0.17	-0.53	-0.31	0.01	-0.38	0.05
10-50% shed leaves (2018)			-0.44	-0.31	0.59	0.34	-	**0.64***	0.42	-0.03	-0.46	0.4	**0.61***	0.5	0.18	0.39	0.09
10-50% shed leaves (2019)	-0.52	-0.65	-0.72	-0.67	0.56	0.05	**0.84***	-	**0.63***	0.18	-0.15	0.27	**0.7****	0.28	0.47	**0.54***	0.51
<50% shed leaves (2018)	-0.41	-0.12	-0.15	-0.16	0.44	0.32	0.57	0.65	-	-0.21	**-0.61***	-0.27	0.17	-0.25	-0.16	0.18	-0.19
<50% shed leaves (2019)	-0.46	-0.11	-0.23	-0.21	0.59	0.4	0.57	0.69	**0.98*****	-	0.38	0.16	0.32	0.24	-0.2	0.35	0.44
LD(i)	-0.7	-0.13	-0.55	-0.39	0.69	0.37	0.28	0.55	0.66	**0.77***	-	0.38	0.2	0.32	0.24	-0.2	0.35
LD (ii)	-0.63	-0.62	**-0.97*****	**-0.76***	0.57	0.02	0.54	0.74	0.24	0.34	0.66	-	**0.84*****	**0.9*****	0.43	0.37	0.29
GP (i)	-0.68	-0.74	**-0.96*****	**-0.85***	0.5	-0.11	0.65	**0.89****	0.37	0.44	0.59	**0.95****	-	**0.81*****	0.5	**0.58***	0.42
GP (ii)	**-0.83***	-0.61	**-0.92****	**-0.9****	0.5	-0.17	0.51	**0.85***	0.44	0.53	0.7	**0.9****	**0.96*****	-	0.4	0.24	0.27
Height	0.02	-0.17	-0.53	-0.37	0.69	0.37	**0.81***	0.6	0.22	0.27	0.25	0.63	0.61	0.48	-	0.41	**0.8*****
Diameter	-0.03	-0.11	-0.35	-0.34	0.58	0.29	**0.87***	0.66	0.52	0.51	0.24	0.43	0.51	0.44	**0.89****	-	0.52
Cr	-0.23	-0.49	-0.3	-0.36	0.34	0.07	**0.84***	0.87*	**0.76***	0.73	0.27	0.32	0.56	0.52	0.43	0.67	-

LD (i): leaf development (i) number of days between “buds visible” and “leaf complete”; LD (ii): leaf development (ii) number of days between “signs of leaves” and “leaf complete”; GP (i): Growing period (i) number of days between “signs of leaves “and “10-50% brown shed leaves”; GP (ii): Growing period (ii) number of days between “signs of leaves” and “>50% brown shed leaves”.

Table S2. Pairwise correlations between leafing phases and climate variables in diploids and tetraploid populations' means (without Sodera and India60)

	Diploid				Tetraploid			
	Temp_mean	Prec_mean	WAI_i	WAI_f	Temp_mean	Prec_mean	WAI_i	WAI_f
Buds visible	0.13	0.14	-0.32	-0.04	0.65	-0.81	0.45	-0.72
Bud developed	0.14	0.04	-0.39	-0.1	0.51	0.12	-0.43	0.21
Signs of leaves	0.19	0.35	**-0.65***	0.12	0.39	-0.34	-0.22	-0.28
Leaflets visible	0.22	0.21	**-0.57***	-0.28	0.65	-0.44	-0.02	-0.46
Leaf expansion	0.27	0.1	-0.28	0.18	-0.38	0.48	-0.46	0.23
Complete leaves	0.39	-0.05	-0.2	0.02	0.44	-0.13	-0.21	-0.31
10-50% shed leaves (2018)	0.06	0.01	0.34	0.11	-0.68	-0.28	0.3	-0.38
10-50% shed leaves (2019)	-0.04	0.36	-0.06	-0.02	-0.84	0.15	-0.04	0.03
>50% shed leaves (2018)	-0.24	0.35	-0.28	-0.05	-0.78	0.54	-0.76	0.36
>50% shed leaves (2019)	-0.21	0.23	-0.03	0.34	-0.75	0.54	-0.74	0.35
Growing period (i)	0.06	-0.13	0.18	0.05	-0.4	0.76	-0.59	0.57
Growing period (ii)	-0.11	-0.35	**0.6***	-0.09	-0.34	0.34	0.2	0.24
Leaf development (i)	-0.18	-0.09	0.45	-0.1	-0.6	0.28	0.14	0.2
Leaf development (ii)	0.03	-0.4	**0.77****	0	-0.7	0.52	-0.13	0.43

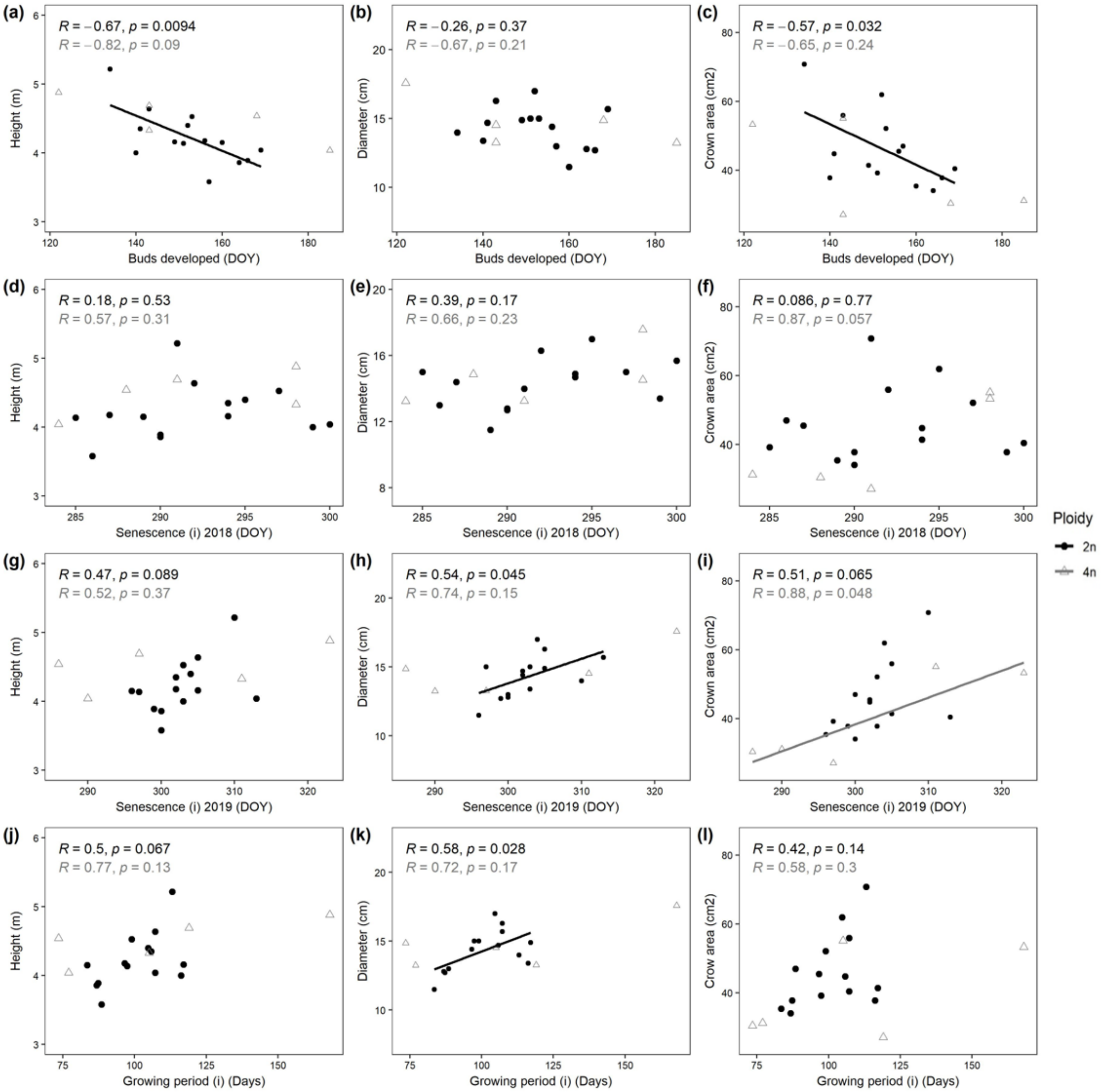

Fig. S3 Pairwise plots of the growth variables (height, diameter and crown area) against bud developed (a, b, c), senescence (i) in 2018 (d, e, f) and 2019 (g, h, i) and the photosynthetic activity (i) (j, k, l) without the populations India and Sodera. Symbols represent population means. Dark symbols denote diploid populations, whereas grey symbols denote tetraploids.