



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

Oulimata Diatta^{1,2} · Erik Dahl Kjær¹ · Adja Madjiguene Diallo² · Lene Rostgaard Nielsen¹ · Vlastimil Novak³ · Diaminatou Sanogo² · Kristian Holst Laursen³ · Jon Kehlet Hansen¹ · Anders Ræbild¹

Received: 13 November 2020 / Accepted: 21 August 2021

© The Author(s) 2021

Abstract

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 ($\delta^{13}\text{C}$) among diploid trees, while a significant correlation was observed with nitrogen isotope composition ($\delta^{15}\text{N}$) among tetraploid trees.

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 the 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 ($\delta^{13}\text{C}$) and nitrogen isotope ratios ($\delta^{15}\text{N}$) 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 $\delta^{13}\text{C}$ and low $\delta^{15}\text{N}$ 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*

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 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).

Communicated by Gessler.

✉ Oulimata Diatta
dou@ign.ku.dk; diatta_oulimata@yahoo.com

✉ Anders Ræbild
are@ign.ku.dk

Extended author information available on the last page of the article

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 CO₂ 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, 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 variation 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, rainfall 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 CO₂ and H₂O 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 N₂-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 ($\delta^{15}\text{N}$) in plants have been used as an indirect measure of N cycling parameters (Craine et al. 2015), and ¹⁵N natural abundance has been used to estimate N₂ fixation in leguminous trees growing naturally or in agroforestry systems (Raddad et al. 2005). The $\delta^{15}\text{N}$ source is preserved during N absorption, assimilation and translocation, and leaf $\delta^{15}\text{N}$ 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 deepwater 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-Saharan 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-Saharan 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 the 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, 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 ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$).

We also compared the leaf morphological variation to the climatic conditions at the place of geographic origin.

Materials and methods

The common garden experiment

We studied *A. senegal* trees in a common garden (provenance) trial in Senegal, established in August 1994 in Dahra (15° 20' N, 15° 28' W, elevation 45 m). 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 °C (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 m. 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 and 29.9 °C (Table 1).

The tree mortality from 1994 to 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.

Determination of the ploidy level

In October 2018, samples of shoot tips with immature leaves from 225 living trees were collected and conserved at 8 °C 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 a 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

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

Population	Country	Latitude	Longitude	Altitude (masl)	Mean annual temperature (°C)*	Annual precipitation (mm)*	Aridity Index
Diamenar	Senegal	16°00'N	15°54'W	20	27.13	288	8
Kankoussa	Mauritania	15°56'N	11°27'W	80	29.71	294	7
Djiguéri	Mauritania	15°44'N	08°40'W	226	28.92	309	8
Chad	Chad	12°49'N	15°18'E	280	28.97	349	9
Karofane	Niger	14°18'N	06°11'E	280	28.38	366	10
Kirane	Mali	15°23'N	10°15'W	140	29.32	380	10
Sudan	Sudan	12°44'N	29°35'E	620	26.84	408	11
Daiba	Senegal	15°22'N	13°08'W	28	29.56	437	11
Aite	Mali	15°05'N	11°39'W	80	29.65	449	11
Ngane	Senegal	14°08'N	16°12'W	2	27.92	570	15
Kidira	Senegal	14°28'N	12°13'W	39	28.93	611	16
Somo	Mali	13°17'N	04°54'W	40	27.45	627	17
Di	Burkina Faso	13°10'N	03°25'W	260	28.02	637	17
Bissiga	Burkina Faso	12°26'N	00°32'W	280	28.28	696	18
Sodera	Ethiopia	08°24'N	39°23'E	1500	21.36	762	24
India60	India	26°19'N	79°31'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 $P/(T+10)$ where P is annual precipitation and T is mean annual temperature

mesh size. After 30–60 min 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×N.A. 0.8 air (Partec).

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).

Carbon and nitrogen stable isotope ratio analysis and nitrogen content quantification

To test for variation in total leaf N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and their relationship to leaf morphology, ploidy and place of origin, several completely healthy-looking leaves including their petioles 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 60 °C for 48 h and grinded with a ball mill. The stable isotope ratios ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) 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 ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) 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 $<0.2\text{‰}$ and $<0.1\text{‰}$ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, respectively.

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

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

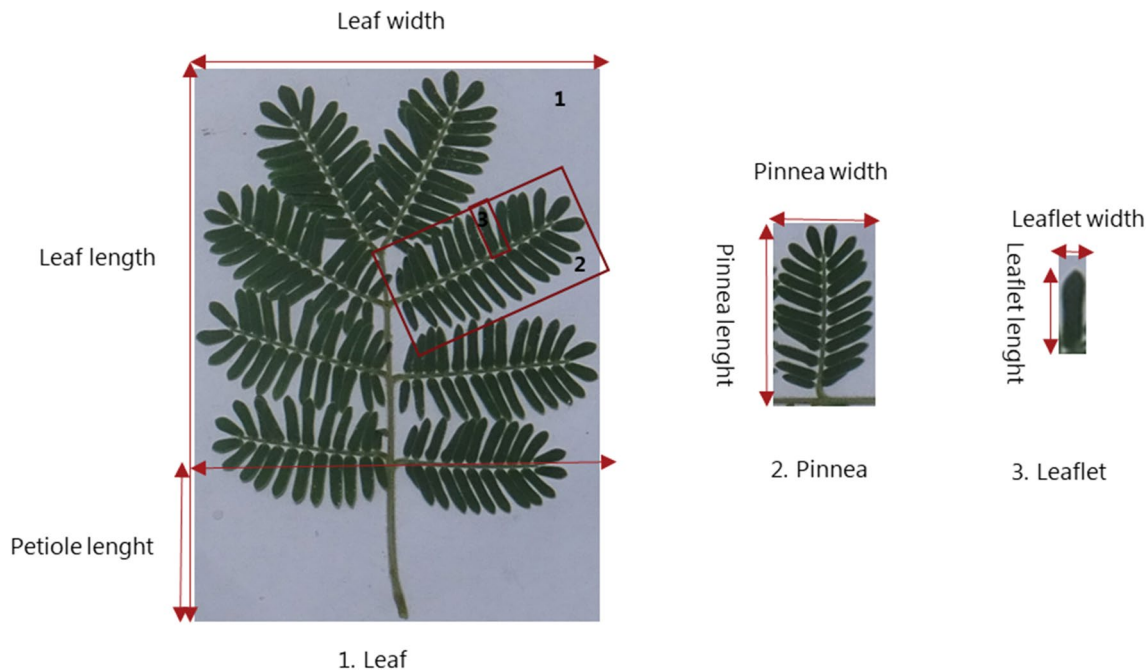


Fig. 1 *Acacia senegal* leaf morphological characters measured

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. $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) 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 $\delta^{13}\text{C}$, and AIR for $\delta^{15}\text{N}$.

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 ($^{15}\text{N}/^{14}\text{N}$) to explore the plant N source and ($^{13}\text{C}/^{12}\text{C}$) as a proxy for WUE—assuming that all plants were exposed to the same CO_2 concentration and composition, and thus variation in carbon isotopic composition reflects intrinsic WUE.

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 tree data from the analyses.

Second, 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, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. 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. 2015a,b). 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 2):

$$Y_{ijk} = \mu + B_i + G_j + P_k + \varepsilon_{ijk} \quad (2)$$

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 3):

$$Y_{ij} = \mu + B_i + G_j + \varepsilon_{ij}, \quad (3)$$

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, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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.

Results

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). Eight 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.

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. mellifera*. 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).

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).

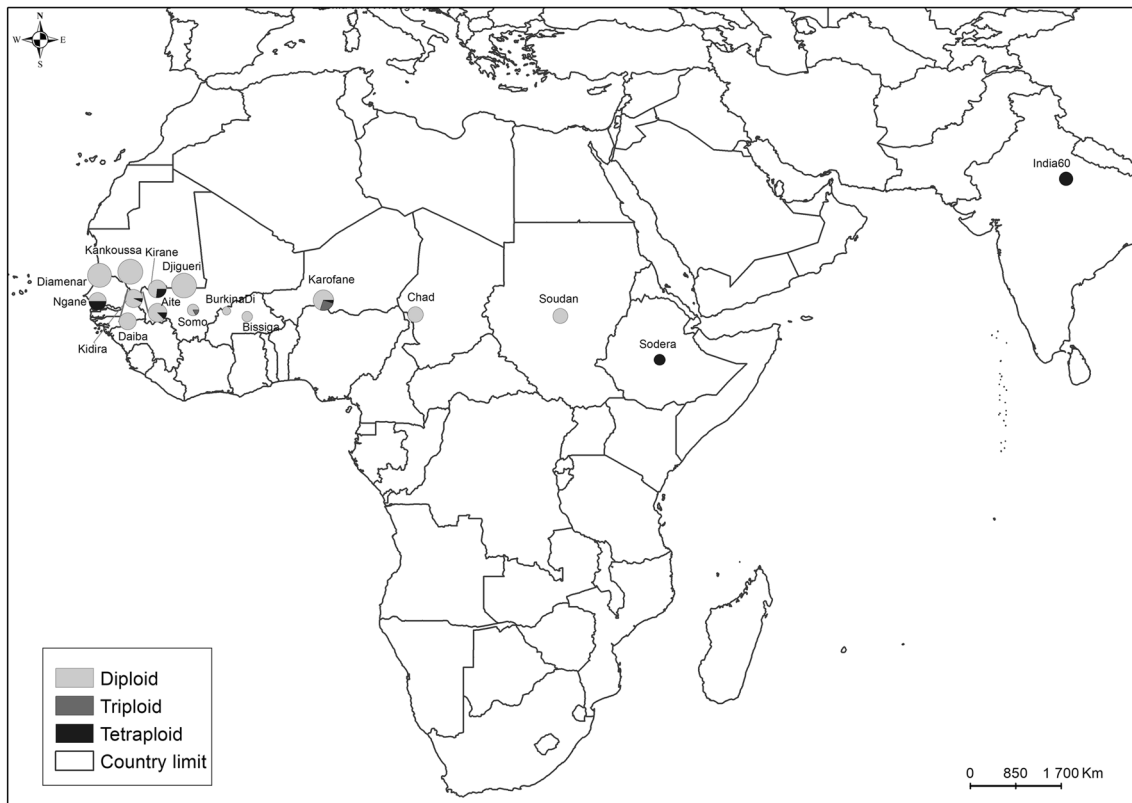


Fig. 2 Distribution of *A. senegal* ploidy levels among the 16 populations located in the common garden in Dahra, Senegal. Circle area and pie represent sample size and relative frequency of ploidy levels

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
Djiguéri	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
Soderia	0	0	6	6
India60	0	0	9	9
Total	189	6	30	225

Variation of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and total leaf N

Diploids and tetraploids displayed highly significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and total leaf N. Tetraploids, which differed from diploids by having longer petioles and leaves, had the highest mean value of $\delta^{13}\text{C}$ and therefore the highest water use efficiency, and the lowest $\delta^{15}\text{N}$ 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).

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

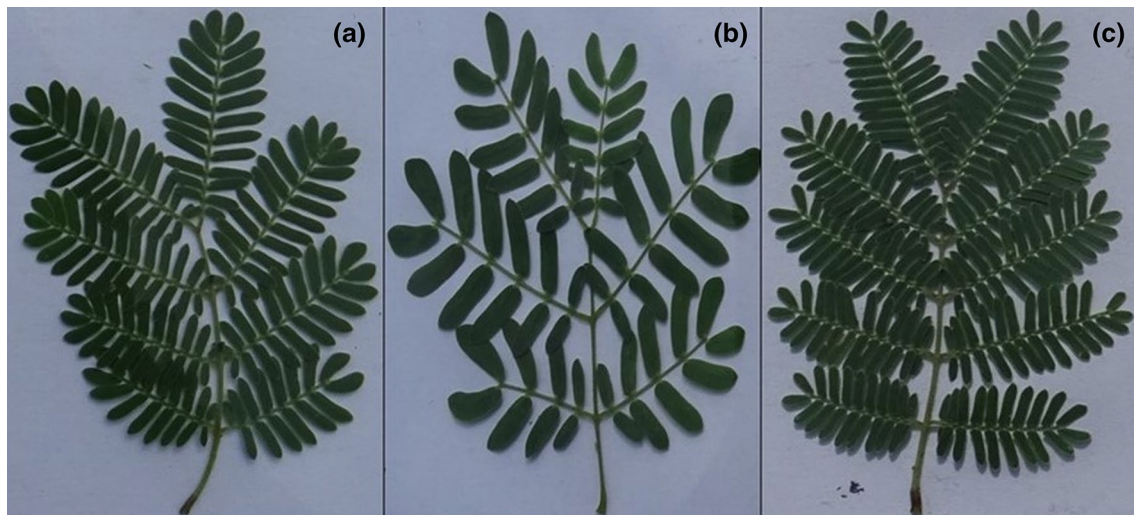


Fig. 3 Diploid (a), Triploid (b) and Tetraploid (c) leaves of *Acacia senegal* from the Karofane population

Table 3 Results from the analysis on leaf morphology, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *Acacia senegal* between diploid and tetraploid trees of *A. senegal* based on data from populations represented by diploid and tetraploid trees (*model 2*) and among diploid and tetraploid populations (*model 3*)

Variables	Mean values				Statistical results								
	Diploid		Tetraploid		Ploidy levels (<i>model 2</i>)			Population tests (<i>model 3</i>)					
		<i>n</i>		<i>n</i>	<i>Df</i>	<i>F</i> value	<i>P</i> value	Diploids			Tetraploids		
								<i>Df</i>	<i>F</i> value	<i>P</i> value	<i>Df</i>	<i>F</i> value	<i>P</i> 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
Pinnae 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
Pinnae 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*
$\delta^{15}\text{N}$ ‰	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
$\delta^{13}\text{C}$ ‰	-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; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

the traits among both diploid and tetraploid populations (Table S4).

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).

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 extent 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

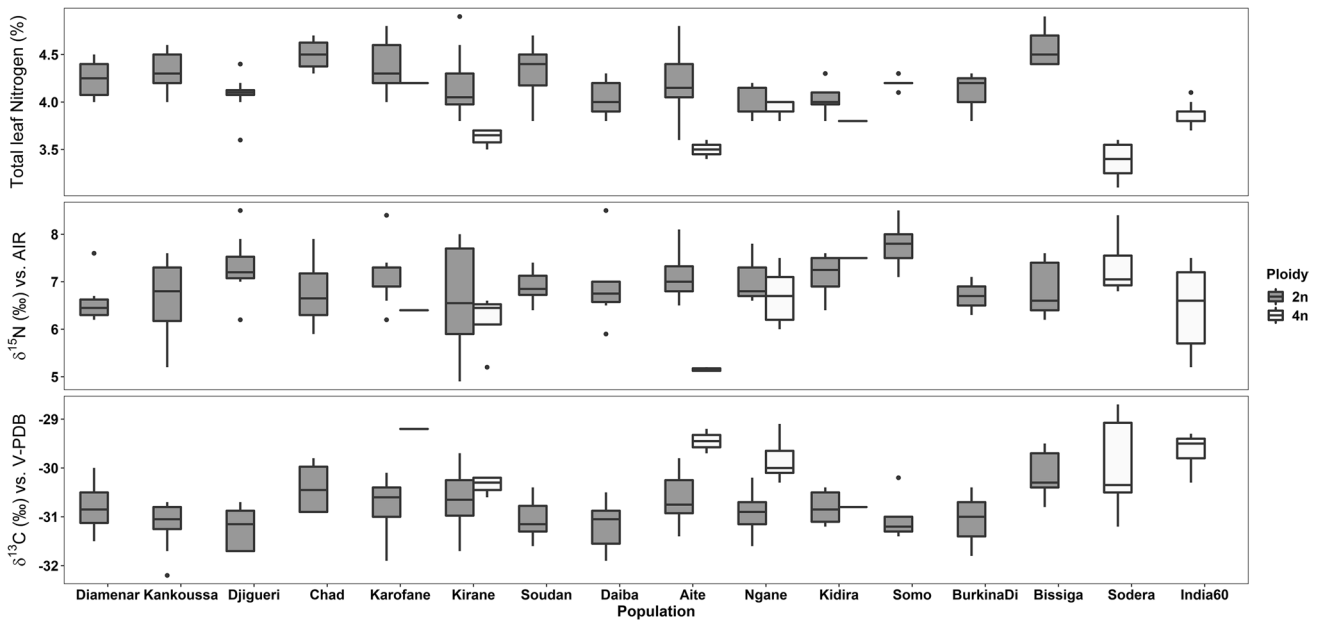


Fig. 4 Box plot of variation in carbon and nitrogen isotope ratios and total leaf nitrogen among populations and ploidy levels. Lines represent populations with only one tree at the ploidy level. Box plots represent median, first-third quartile, minimum and maximum values. Gray and white boxes represent diploid and tetraploid trees, respec-

tively. 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 $\delta^{15}\text{N}$ (both ploidy levels) and for $\delta^{13}\text{C}$ (tetraploids)

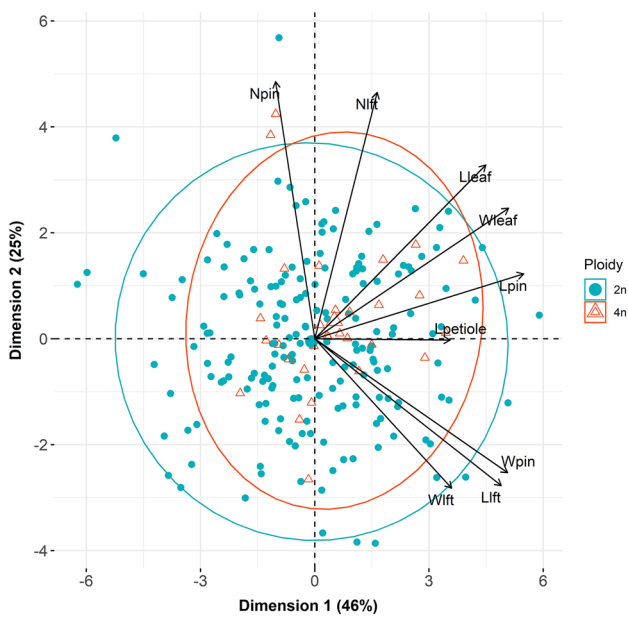


Fig. 5 Plot of the principal components one and two among ploidy level. The directions of the arrows show the relative loadings of the leaf traits. *Npin* number of pinnae per leaf, *Nlft* number of leaflets per leaf, *Lpetiole* petiole length, *Lleaf* leaf length, *Wleaf* leaf width, *Lpin* pinnae length,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

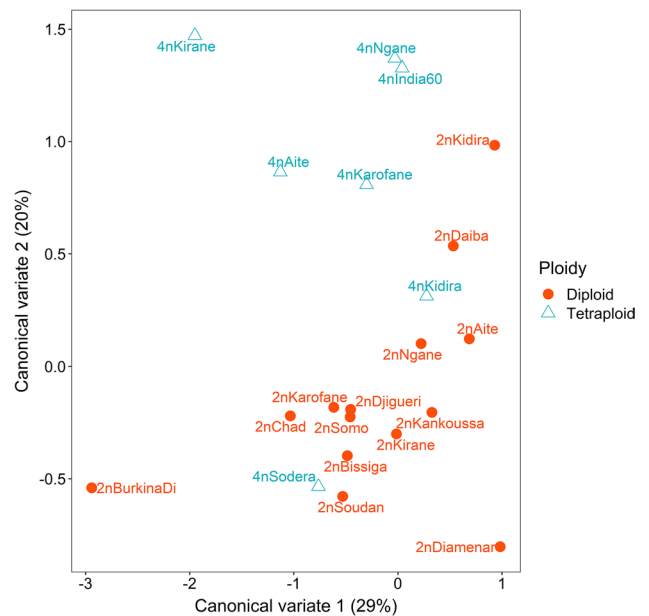


Fig. 6 Canonical scores of the diploid and tetraploid populations, plotted against the two first canonical variates

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).

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 the 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).

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, $\delta^{13}\text{C}$ was positively correlated with the length and width of the leaf and the pinnae length, and $\delta^{15}\text{N}$ increased with the length of the petiole. Conversely, in tetraploid populations, $\delta^{13}\text{C}$ was negatively correlated with petiole length, leaf width, and pinnae length and width. $\delta^{15}\text{N}$ was negatively correlated with the number of pinnae and positively correlated with the leaflet width. Hence, tendencies of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among 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).

Discussion

Geographic distribution of polyploidy among populations

The present study documents the 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 the 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 previously reported a high frequency of polyploids (Diallo et al. 2015) which supports our findings.

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 pinnae having at least three to five widely elliptical leaflets. One hypothesis is that the triploid individuals observed in this study have a hybrid origin between *A. senegal* var. *senegal* and *A. mellifera* subsp. *mellifera*, a hybrid first recognized by Kan (1951) (hereafter 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 \times 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.

Table 4 Pairwise correlations given by Pearson's correlation coefficient at the population means between leaf measured traits, geographical parameters and isotope ratios ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) 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	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	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
Annual Temp_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	-	-0.77**	-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*
$\delta^{13}\text{C}$	-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**
$\delta^{15}\text{N}$	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

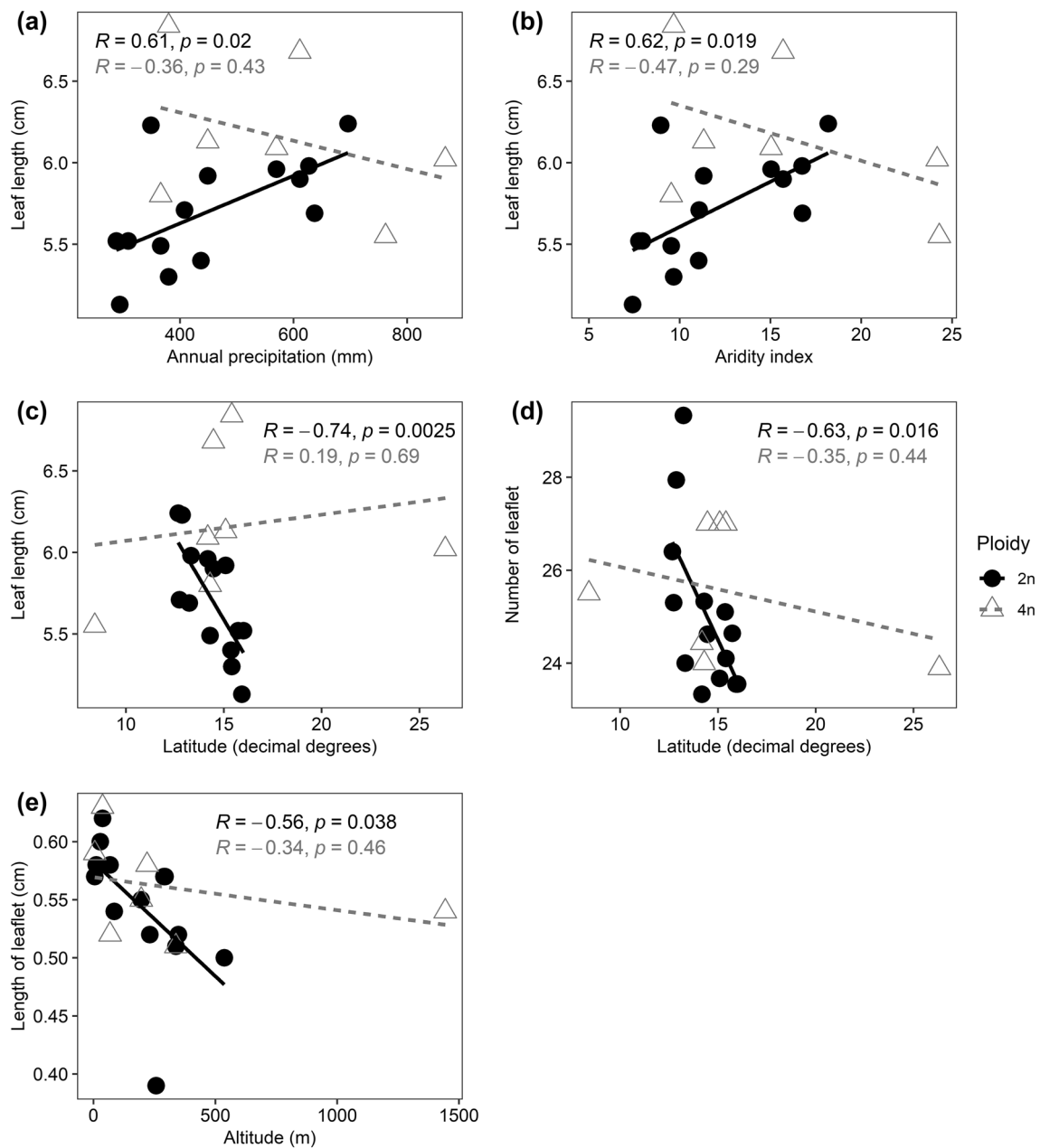


Fig. 7 Pairwise plots of, leaf length against annual precipitation (a) and aridity index (b), and latitude (c), and length of leaflet against latitude (c), and length of leaflet against altitude (e) in diploid and tetraploid populations. Symbols represent population means

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. Other Sahelian tree species also show variation in leaf morphology between origins, but observations made in *Parkia*

biglobosa showed that the variation was rather across an east–west gradient (Ouedraogo 2012), whereas in *Adansonia digitata* (Cuni Sanchez et al. 2010; Korbo et al. 2011) variation was mainly between a western pool of origins and a south-eastern pool.

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

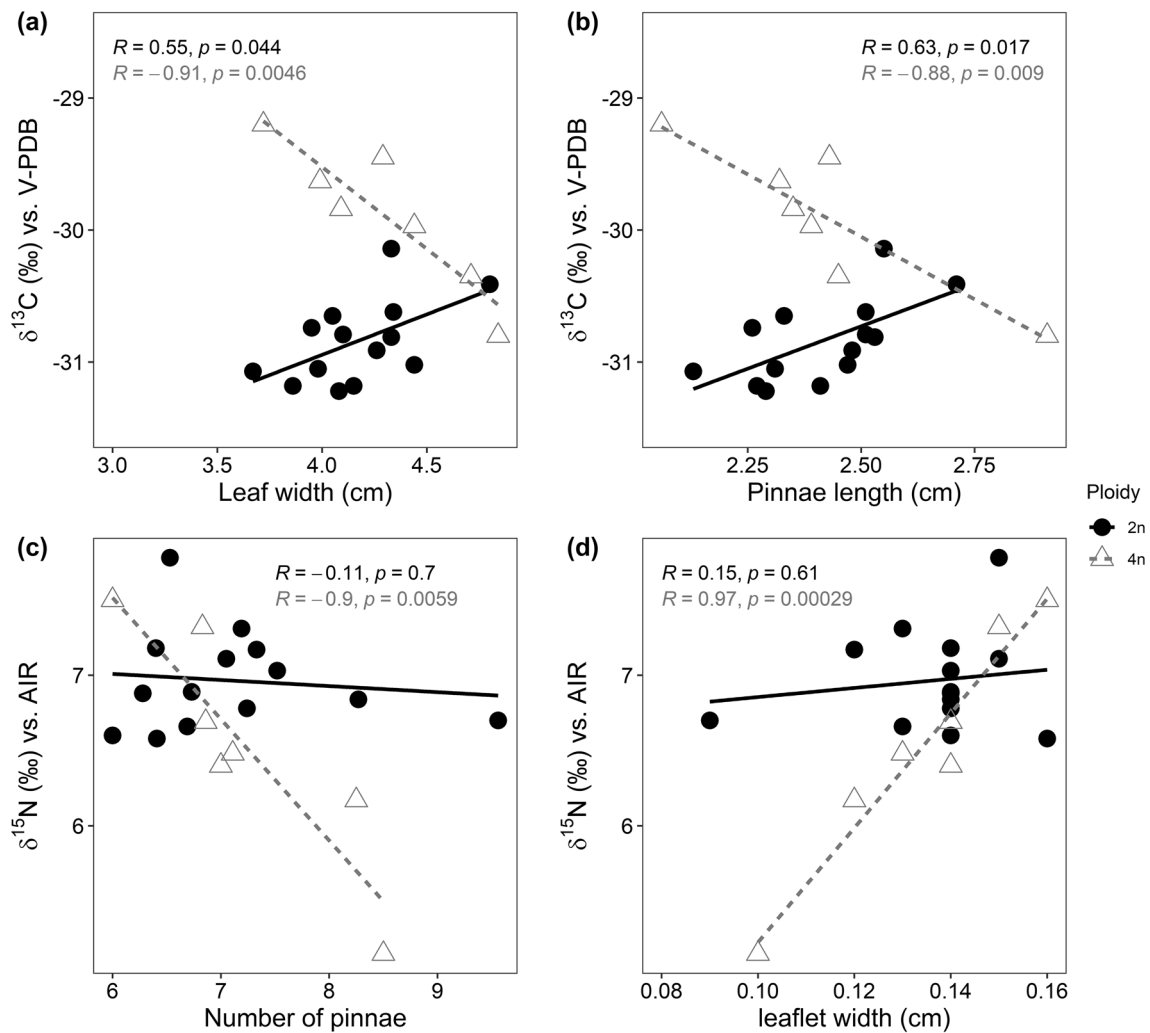


Fig. 8 Pairwise plots of the stable carbon isotope composition against leaf width (a) and pinnae length (b) and nitrogen isotope ratio against number of pinnae (c) and leaflet width (d) in diploid and tetraploid populations. Symbols represent population means

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 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 a result of a 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 $\delta^{15}\text{N}$. While this could indicate that diploids and tetraploids differ in the sourcing of nitrogen, the small differences in mean values between ploidy levels call for additional studies. The $\delta^{15}\text{N}$ values of leaves reflect $^{15}\text{N}/^{14}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ values for diploids (6.9 ± 0.68) and tetraploids (6.6 ± 0.83) are close to the values found by Gray et al. (2013) at the same site, considering that Gray et al. 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. and the study presented here represent a limited sample of tetraploid individuals, and further investigations will be needed to clarify the implication of polyploidy 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 $\delta^{13}\text{C}$ and geographic origin in diploid populations. A weakness in our study is that isotopic composition and leaf morphology were studied on two different sets of leaves, but since most leaves in the open crown of *A. senegal* are sun exposed, we believe that this has less influence (see below). 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ and iWUE were correlated to stomatal conductance under saturating light conditions, suggesting an important role for stomata. However, the measured differences in $\delta^{13}\text{C}$ 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. In addition, the leaf sampling for isotopic composition was not standardized to only fully sunlit leaves. In dense plantations this would mean that dominated individuals had a higher percentage of shaded leaves, changing the $\delta^{13}\text{C}$ (usually towards more negative values, possibly due to lower photosynthesis for the same stomatal opening). In the current trial, however, trees were placed at a large distance from each other, leaving all trees sun-exposed and minimizing risks for such bias.

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, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ 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 happening in *A. senegal* will require additional studies on adaptive traits in combination with genomic studies.

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 address adaptation of *A. senegal* to dry growing conditions.

Author contribution statement 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.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00468-021-02206-8>.

Acknowledgements We are thankful to Cheikh Omar Samb and Ibra Padane from Institut Sénégalais de Recherches Agricoles/Centre National de Recherches Forestières (ISRA/CNRF) for the technical support during the field work in Senegal, to Robyn Margaret Stuart from the Department of Mathematics at the University of Copenhagen for statistical advice, to Sophia Groll for help with sample preparation in the laboratory of University of Copenhagen, and to the anonymous reviewer for important comments and suggestions.

Funding This study was funded by the Islamic Development Bank (IDB) under the PhD Merit Scholarship program (Student Grant Number 600032772) and by the University of Copenhagen, Denmark.

Data availability Data is publicly available through the University of Copenhagen—Electronic Research Data Archive (ERDA) <https://erda.ku.dk/archives/73e5cd9b4efe3d726b3a8fa0e6d563b8/published-archi-ve.html>.

Declarations

Conflicts of interest The authors declare that they have no conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Abrams MD, Kloeppel BD, Kubiske ME (1992) Ecophysiological and morphological responses to shade and drought in two contrasting ecotypes of *Prunus serotina*. *Tree Physiol* 10(4):343–355. <https://doi.org/10.1093/treephys/10.4.343>
- Abrams MD, Kubiske ME, Steiner KC (1990). Drought adaptations and responses in five genotypes of *Fraxinus pennsylvanica* Marsh.: photosynthesis, water relations and leaf morphology. *Tree Physiol* 6(3): 305–315. <https://doi.org/10.1093/treephys/6.3.305>
- Anderson JE, Williams J, Kriedemann PE, Austin MP, Farquhar GD (1996) Correlations between carbon isotope discrimination and climate of native habitats for diverse *eucalypt* taxa growing in a common garden. *Aust J Plant Physiol* 23(3):311–320. <https://doi.org/10.1071/PP9960311>
- Arntz MA, Delph LF (2001) Pattern and process: evidence for the evolution of photosynthetic traits in natural populations. *Oecologia* 127(4):455–467. <https://doi.org/10.1007/s004420100650>
- Arumuganathan K, Earle ED (1991) Estimation of nuclear DNA content of plants by flow cytometry. *Plant Mol Biol Rep* 9(3):229–241. <https://doi.org/10.1007/BF02672073>
- Assoumane A, Zoubeirou AM, Rodier-Goud M, Favreau B, Bezaçon G, Verhaegen D (2012). Highlighting the occurrence of tetraploidy in *Acacia senegal* (L.) Willd. and genetic variation patterns in its natural range revealed by DNA microsatellite markers. *Tree Genet and Genomes* 9(1): 93–106. <https://doi.org/10.1007/s11295-012-0537-0>
- Baker RL, Yarkhunova Y, Vidal K, Ewers BE, Weinig C (2017) Polyploidy and the relationship between leaf structure and function: Implications for correlated evolution of anatomy, morphology, and physiology in Brassica. *BMC Plant Biol* 17(1):1–12. <https://doi.org/10.1186/s12870-016-0957-3>
- Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67(1):1–48. <https://doi.org/10.18637/jss.v067.i01>
- Battipaglia G, Saurer M, Cherubini P, Calfapietra C, McCarthy HR, Norby RJ, Cotrufo MF (2012) Elevated CO₂ increases tree-level intrinsic water use efficiency: insights from carbon and oxygen

- isotope analyses in tree rings across three forest FACE sites. *New Phytol* 2013(197):544–554. <https://doi.org/10.1111/nph.12044>
- Brandt M, Grau T, Mbow C, Samimi C (2014) Modeling soil and woody vegetation in the senegalese sahel in the context of environmental change. *Land* 3(3):770–792. <https://doi.org/10.3390/land3030770>
- Chansler MT, Ferguson CJ, Fehlberg SD, Prather LA (2016) The role of polyploidy in shaping morphological diversity in natural populations of *Phlox amabilis*. *Am J Bot* 103(9):1546–1558. <https://doi.org/10.3732/ajb.1600183>
- Chevalier MH, Borgel A (1998) Diversité génétique des *acacias*. In: Campa C, Grignon C, Gueye M, Hamon S (eds) *L'acacia au Sénégal*. ORSTOM, Paris, pp 287–308
- Comstock JP, Ehleringer JR (1992). Correlating genetic variation in carbon isotopic composition with complex climatic gradients. *Proc. Natl Acad Sci USA* 89(16):_ 7747–7751. <https://doi.org/10.1073/pnas.89.16.7747>
- Cramer MD, Chimphango SBM, Van Cauter A, Waldram MS, Bond WJ (2007) Grass competition induces N₂ fixation in some species of African *Acacia*. *J Ecol* 95(5):1123–1133. <https://doi.org/10.1111/j.1365-2745.2007.01285.x>
- Craufurd PQ, Wheeler TR, Ellis RH, Summerfield RJ, Williams JH (1999) Effect of temperature and water deficit on water-use efficiency, carbon isotope discrimination, and specific leaf area in peanut. *Crop Sci* 39(1):136–142. <https://doi.org/10.2135/cropsci1999.0011183X003900010022x>
- Cuni Sanchez A, Haq N, Assogbadjo AE (2010). Variation in baobab (*Adansonia digitata* L.) leaf morphology and its relation to drought tolerance. *Genet Resour Crop Ev*, 57(1): 17–25. <https://doi.org/10.1007/s10722-009-9447-x>
- De Baerdemaeker NJF, Hias N, Van den Bulcke J, Keulemans W, Steppe K (2018) The effect of polyploidization on tree hydraulic functioning. *Am J Bot* 105(2):161–171. <https://doi.org/10.1002/ajb2.1032>
- Diallo AM, Nielsen LR, Kjør ED, Petersen KK, Ræbild A (2016) Polyploidy can Confer Superiority to West African *Acacia senegal* (L.) Willd. *Trees Front Plant Sci* 7:1–10. <https://doi.org/10.3389/fpls.2016.00821>
- Diallo AM, Nielsen LR, Hansen JK, Ræbild A, Kjør ED (2015). Study of quantitative genetics of gum arabic production complicated by variability in ploidy level of *Acacia senegal* (L.) Willd. *Tree Genet Genomes* 11(4). <https://doi.org/10.1007/s11295-015-0902-x>
- Du ZY, Yang CF, Chen JM, Guo YH, Kadiri AB (2010) Using dna-based techniques to identify hybrids among linear-leaved potatogeton plants collected in China. *J Syst Evol* 48(4):265–270. <https://doi.org/10.1111/j.1759-6831.2010.00089.x>
- Dupuy NC, Dreyfus BL (1992) *Bradyrhizobium* populations occur in deep soil under the leguminous tree *Acacia albida*. *Appl Environ Microbiol* 58(8):2415–2419
- Evans RD (2001) Physiological mechanisms influencing plant nitrogen isotope composition. *Trends Plant Sci* 6(3):121–126. [https://doi.org/10.1016/S1360-1385\(01\)01889-1](https://doi.org/10.1016/S1360-1385(01)01889-1)
- Fagg C, Allison G (2004). *Acacia senegal* and the Gum Arabic Trade. Monograph and Annotated Bibliography. Tropical Forestry Papers No. 42. Oxford, UK: Oxford Forestry
- Fick SE, Hijmans RJ (2017). Worldclim 2: New 1-km spatial resolution climate surfaces for global land areas. *Int J Climatol*
- Giffard PL (1966) Les gommiers: *Acacia senegal* Wild. *Acacia laeta* R. Br (Gum-trees: *Acacia senegal*, *Acacia laeta*). *Revue Bois Et Forêts Des Tropiques* 105:21–31
- Gilmour AR, Gogel BJ, Cullis BR, Welham SJ, Thompson R (2015). ASReml User Guide Release 4.1 Functional Specification, VSN International Ltd, Hemel Hempstead, HP1 1ES, UK www.vsnl.co.uk
- Gilmour A, Gogel B, Cullis B, Welham S, Thompson R (2015). ASReml User Guide Release 4.1 Structural Specification. Hemel Hempstead: VSN International Ltd.
- Gonzalez P (2001) Desertification and a shift of forest species in the West African Sahel. *Clim Res* 17:217–228 ((in press))
- Göttsche FM, Olesen FS, Trigo IF, Bork-Unkelbach A, Martin MA (2016). Long term validation of land surface temperature retrieved from MSG/SEVIRI with continuous in-situ measurements in Africa. *Remote Sensing*, 8(5). <https://doi.org/10.3390/rs8050410>
- Gray A, Odee D, Cavers S, Wilson J, Telford A, Grant F, Diouf M, Ochieng J, Grant H, Stott A (2013) Does geographic origin dictate ecological strategies in *Acacia senegal* (L.) Willd.? Evidence from carbon and nitrogen stable isotopes. *Plant Soil* 369(1–2): 479–496. <https://doi.org/10.1007/s11104-013-1593-4>
- Greer BT, Still C, Cullinan GL, Brooks JR, Meinzer FC (2018) Polyploidy influences plant–environment interactions in quaking aspen (*Populus tremuloides* Michx.). *Tree Physiol*, 38(4):630–640. <https://doi.org/10.1093/treephys/tpx120>
- Guet J, Fabbri F, Fichot R, Sabatti M, Bastien C, Brignolas F (2015). Genetic variation for leaf morphology, leaf structure and leaf carbon isotope discrimination in European populations of black poplar (*Populus nigra* L.). *Tree Physiol*, 35(8):850–863. <https://doi.org/10.1093/treephys/tpv056>
- Halloy SRP, Mark AF (1996) Comparative leaf morphology spectra of plant communities in New Zealand, the Andes and the European Alps. *J Exp Bot* 26(1):41–78. <https://doi.org/10.1080/03014223.1996.9517504>
- Hultine KR, Marshall JD (2000) Altitude trends in conifer leaf morphology and stable carbon isotope composition. *Oecologia* 123(1):32–40. <https://doi.org/10.1007/s004420050986>
- IPCC (2019) Summary for Policymakers. In: Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems [Shukla PR, Skea J, Calvo Buendia E, Masson-Delmotte V, Pörtner HO, Roberts DC, Zhai P, Slade R, Connors S, van Diemen R, Ferrat M, Haughey E, Luz S, Neogi S, Pathak M, Petzold J, Portugal Pereira J, Vyas P, Huntley E, Kissick K, Belkacemi M, Malley J, (eds.)]. In press
- Kassambara A, Mundt F (2020). factoextra: extract and visualize the results of multivariate data analyses. R package version 1.0.7. <https://CRAN.R-project.org/package=factoextra>
- Khan HR, Paull JG, Siddique KHM, Stoddard FL (2010) Faba bean breeding for drought-affected environments: a physiological and agronomic perspective. *Field Crops Res* 115(3):279–286. <https://doi.org/10.1016/j.fcr.2009.09.003>
- Khrantsova EV, Kiseleva IS, Lyubomudrova EA, Malkova NV (2003) Optimization of the leaf mesophyll structure in allopolyploid and diploid wheat species. *Russ J Plant Physiol* 50(1):19–27. <https://doi.org/10.1023/A:1021924115326>
- Koksheeva I, Kislov D, Tvorogov S, Doudkin R (2017) Relationships between leaf shape and climate in *Rhododendron mucronulatum*. *Nord J Bot* 35(5):618–626. <https://doi.org/10.1111/njb.01486>
- Konate NM, Dreyer E, Epron D (2016) Differences in carbon isotope discrimination and whole-plant transpiration efficiency among nine Australian and Sahelian *Acacia* species. *Ann for Sci* 73(4):995–1003. <https://doi.org/10.1007/s13595-016-0589-7>
- Korbo A, Sanou H, Ræbild A, Jensen JS, Hansen JK, Kjør ED (2012). Comparison of East and West African populations of baobab (*Adansonia digitata* L.). *Agrofor Syst* 85(3): 505–518. <https://doi.org/10.1007/s10457-011-9464-6>
- Körner C, Farquhar GD, Wong SC (1991) Carbon isotope discrimination by plants follows latitudinal and altitudinal trends. *Oecologia* 88(1):30–40. <https://doi.org/10.1007/BF00328400>

- Lambers H, Chapin III FS, Pons TL (2008). Plant Physiological Ecology, 2nd edn, vol 53. Springer <https://doi.org/10.1017/CBO9781107415324.004>
- Larwanou M, Raebild A, Issa R, Kjær ED (2010). Performance of *Acacia senegal* (L.) Willd Provenances in Dryland Savannah of Niger. *Silvae Genet* 59(5): 210–218. <https://doi.org/10.1515/sg-2010-0025>
- Li WL, Berlyn GP, Ashton PMS (1996) Polyploids and their structural and physiological characteristics relative to water deficit in *Betula papyrifera* (Betulaceae). *Am J Bot* 83(1):15–20. <https://doi.org/10.2307/2445949>
- Li WD, Biswas DK, Xu H, Xu CQ, Wang XZ, Liu JK, Jiang GM (2009) Photosynthetic responses to chromosome doubling in relation to leaf anatomy in *Lonicera japonica* subjected to water stress. *Funct Plant Biol* 36(9):783–792. <https://doi.org/10.1071/FP09022>
- Lu Z, Chen J, Percy RG, Zeiger E (1997) Photosynthetic rate, stomatal conductance and leaf area in two cotton species (*Gossypium barbadense* and *Gossypium hirsutum*) and their relation with heat resistance and yield. *Aust J Plant Physiol* 24(5):693–700. <https://doi.org/10.1071/PP97056>
- Maherali H, Walden AE, Husband BC (2009) Genome duplication and the evolution of physiological responses to water stress. *New Phytol* 184(3):721–731. <https://doi.org/10.1111/j.1469-8137.2009.02997.x>
- Manzaneda AJ, Rey PJ, Bastida JM, Weiss-Lehman C, Raskin E, Mitchell-Olds T (2012) Environmental aridity is associated with cytotype segregation and polyploidy occurrence in *Brachypodium distachyon* (Poaceae). *New Phytol* 193(3):797–805. <https://doi.org/10.1111/j.1469-8137.2011.03988.x>
- McDonald PG, Fonseca CR, Overton JMC, Westoby M (2003) Leaf-size divergence along rainfall and soil-nutrient gradients: Is the method of size reduction common among clades? *Funct Ecol* 17(1):50–57. <https://doi.org/10.1046/j.1365-2435.2003.00698.x>
- McKey D (1994) Legumes and nitrogen : the evolutionary ecology of a nitrogen-demand ing lifestyle. In: Sprent JI, McKey D, eds. *Advances in Legume Systematics*. 5. The Nitrogen Factor. Kew: Royal Botanic Gardens, p 211–228
- Mensous M, Van de Paer C, Manzi S, Bouchez O, Baâli-Cherif D, Besnard G (2017). Diversity and evolution of plastomes in Saharan mimosoids: potential use for phylogenetic and population genetic studies. *Tree Genet Genomes* 13(2). <https://doi.org/10.1007/s11295-017-1131-2>
- Ndoye F, Kane A, Ngonkeu MEL, Bakhoun N, Sanon A, Diouf D, Sy MO, Baudoin E, Noba K, Prin Y (2012) Changes in land use system and environmental factors affect arbuscular mycorrhizal fungal density and diversity, and enzyme activities in rhizospheric soils of *Acacia senegal* (L.) Willd. *ISRN Ecology* 2012:1–13. <https://doi.org/10.5402/2012/563191>
- Novak V, Adler J, Husted S, Fromberg A, Laursen KH (2019) Authenticity testing of organically grown vegetables by stable isotope ratio analysis of oxygen in plant-derived sulphate. *Food Chem* 291:59–67. <https://doi.org/10.1016/j.foodchem.2019.03.125>
- Odee DW, Telford A, Wilson J, Gaye A, Cavers S (2012) Plio-Pleistocene history and phylogeography of *Acacia senegal* in dry woodlands and savannahs of sub-Saharan tropical Africa: evidence of early colonisation and recent range expansion. *Heredity* 109(6):372–382. <https://doi.org/10.1038/hdy.2012.52>
- Odee DW, Wilson J, Omondi S, Perry A, Cavers S (2015). Rangewide ploidy variation and evolution in *Acacia senegal*: A north-south divide?. *AoB Plants* 7(1). <https://doi.org/10.1093/aobpla/plv011>
- Omondi SF, Odee DW, Ongamo GO, Kanya JI, Khasa DP (2018) Mating patterns of the gum arabic tree (*Acacia senegal* synonym *Senegalia senegal*) in two different habitats. *New for* 49(1):53–65. <https://doi.org/10.1007/s11056-017-9604-6>
- Otieno DO, Schmidt MWT, Adiku S, Tenhunen J (2005) Physiological and morphological responses to water stress in two *Acacia* species from contrasting habitats. *Tree Physiol* 25(3):361–371. <https://doi.org/10.1093/treephys/25.3.361>
- Ouedraogo M (2015). Improving and conserving sahelian fruits trees : a case study of *Parkia biglobosa* (Jacq .) Benth. Dissertation, Department of Geosciences and Natural Resource Management, Faculty of Science, University of Copenhagen.
- Peppe DJ, Royer DL, Cariglino B, Oliver SY, Leight E, Enikolopov G, Fernandez-burgos M, Herrera F, Adams JM, Correa E, Curran ED, Erickson JM, Felipe L, Hoganson JW, Iglesias A, Jaramillo CA, Johnson KR, Jordan J, Kraft NJB, Lovelock EC (2011) Sensitivity of leaf size and shape to climat patterns and paleoclimat. *New Phytol* 190(3):724–739. <https://doi.org/10.1111/j.1469-8137.2010.03615.x>
- R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>
- Raddad EAY, Luukkanen O (2006) Adaptive genetic variation in water-use efficiency and gum yield in *Acacia senegal* provenances grown on clay soil in the Blue Nile region. *Sudan for Ecol Manage* 226(1–3):219–229. <https://doi.org/10.1016/j.foreco.2006.01.036>
- Raddad EAY, Salih AA, Fadl MAE, Kaarakka V, Luukkanen O (2005) Symbiotic nitrogen fixation in eight *Acacia senegal* provenances in dryland clays of the Blue Nile Sudan estimated by the 15N natural abundance method. *Plant Soil* 275(1–2):261–269. <https://doi.org/10.1007/s1104-005-2152-4>
- Ræbild A, Larsen AS, Jensen JS, Ouedraogo M, de Groot S, van Damme P, Bayala J, Diallo BO, Sanou H, Kalinganire A, Kjær ED (2010) Advances in domestication of indigenous fruit trees in the West African Sahel. *New for* 41(3):297–315. <https://doi.org/10.1007/s11056-010-9237-5>
- Ræbild A, Graudal L, Ouedraogo LG (2003a). Evaluation of a provenance trial of *Acacia senegal* at Gonsé, Burkina Faso. Trial no. 12 in the arid zone series. Results and Documentation No 5. Danida Forest Seed Centre, Humlebaek, Denmark
- Ræbild A, Graudal L, Cesar P, Lima F (2003b). Evaluation of a provenance trial of *Acacia senegal* at Dori, Burkina Faso: Trial no. 8 in the arid zones series. DFSC Results and Documentation No 3, Danida Forest Seed Centre, Humlebaek, Denmark
- Ræbild A, Diallo BO, Graudal L, Dao M and Sanou J (2003c). Evaluation of a provenance trial of *Acacia senegal* at Djibo, Burkina Faso. Trial no. 5 in the arid zone series. Results and Documentation No 7. Danida Forest Seed Centre, Humlebaek, Denmark
- Ramsey J, Ramsey TS (2014) Ecological studies of polyploidy in the 100 years following its discovery. *Phil Trans R Soc B* 369(1648):15–19. <https://doi.org/10.1098/rstb.2013.0352>
- Ramsey J, Schemske DW (1998) Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annu Rev Ecol and Syst* 29:467–501. <https://doi.org/10.1146/annurev.ecolsys.29.1.467>
- Revelle W (2019). psych: Procedures for Personality and Psychological Research, Northwestern University, Evanston, Illinois, USA, <https://CRAN.R-project.org/package=psych> Version = 1.9.12
- Royer DL, McElwain JC, Adams JM, Wilf P (2008) Sensitivity of leaf size and shape to climate within *Acer rubrum* and *Quercus kelloggii*. *New Phytol* 179(3):808–817. <https://doi.org/10.1111/j.1469-8137.2008.02496.x>
- Sarr MS, Seiler JR, Sullivan J, Diallo AM, Strahm BD (2021). Drought resistance and gum yield performances in a *Senegalia senegal* (L.) Britton progeny trial in Senegal. *New Forests*, 0123456789. <https://doi.org/10.1007/s11056-020-09825-y>
- SAS Institute Inc (2004) SAS/STAT Users' Guide. SAS Institute Inc., Cary
- Schmerler SB, Clement WL, Beaulieu JM, Chatelet DS, Sack L, Donoghue MJ, Edwards EJ, Schmerler SB, Clement WL, Beaulieu JM, Chatelet DS, Sack L, Donoghue MJ, Edwards EJ (2012) JSTOR: Evolution of leaf form correlates with tropical

- temperate transitions in *Viburnum* (*Adoxaceae*). *Proc R Soc B* 279(1744):3905–3913. <https://doi.org/10.1098/rspb.2012.11>
- Schuepp PH (1993) Leaf boundary layers. *New Phytol* 125:477–507
- Seibt U, Rajabi A, Griffiths H, Berry JA (2008) Carbon isotopes and water use efficiency: sense and sensitivity. *Oecologia* 155(3):441–454. <https://doi.org/10.1007/s00442-007-0932-7>
- Soltis DE, Visger CJ, Soltis PS (2014). The polyploidy revolution then...and now: Stebbins revisited. *Am J Bot* 101(7): 1057–1078. <https://doi.org/10.3732/ajb.1400178>
- Stanhill G (1986). Water use efficiency. *Adv Agron*, 39(C), 53–85. [https://doi.org/10.1016/S0065-2113\(08\)60465-4](https://doi.org/10.1016/S0065-2113(08)60465-4)
- Teklehaimanot Z, Lanek J, Tomlinson HF (1998) Provenance variation in morphology and leaflet anatomy of *Parkia biglobosa* and its relation to drought tolerance. *Trees* 13(2):96–102. <https://doi.org/10.1007/s004680050192>
- Traiser C, Klotz S, Uhl D, Mosbrugger V (2005) Environmental signals from leaves—a physiognomic analysis of European vegetation. *New Phytol* 166(2):465–484. <https://doi.org/10.1111/j.1469-8137.2005.01316.x>
- Turchetto C, Segatto ALA, Beduschi J, Bonatto SL, Freitas LB (2015) Genetic differentiation and hybrid identification using microsatellite markers in closely related wild species. *AoB Plants*. <https://doi.org/10.1093/aobpla/plv084>
- Vincke C, Diédhiou I, Grouzis M (2009) Long term dynamics and structure of woody vegetation in the Ferlo (Senegal). *J Arid Environ* 74(2):268–276. <https://doi.org/10.1016/j.jaridenv.2009.08.006>
- Wickham H (2016) *ggplot2: elegant graphics for data analysis*. Springer-Verlag, New York
- Xu Z, Zhou G (2008) Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *J Exp Bot* 59(12):3317–3325. <https://doi.org/10.1093/jxb/ern185>
- Zhang F, Xue H, Lu X, Zhang B, Wang F, Ma Y, Zhang Z (2015) Autotetraploidization enhances drought stress tolerance in two apple cultivars. *Trees* 29(6):1773–1780. <https://doi.org/10.1007/s00468-015-1258-4>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Authors and Affiliations

Oulimata Diatta^{1,2}  · Erik Dahl Kjær¹ · Adja Madjiguene Diallo² · Lene Rostgaard Nielsen¹ · Vlastimil Novak³ · Diaminatou Sanogo² · Kristian Holst Laursen³ · Jon Kehlet Hansen¹ · Anders Ræbild¹

Erik Dahl Kjær
edk@ign.ku.dk

Adja Madjiguene Diallo
madjidiallo@yahoo.fr

Lene Rostgaard Nielsen
Iron@ign.ku.dk

Vlastimil Novak
vln@plen.ku.dk

Diaminatou Sanogo
sdiami@yahoo.fr

Kristian Holst Laursen
holst@plen.ku.dk

Jon Kehlet Hansen
jkh@ign.ku.dk

¹ Department of Geosciences and Natural Resource Management, University of Copenhagen, Rolighedsvej 23, 1958 Frederiksberg C, Denmark

² Institut Sénégalais de Recherches Agricoles/Centre National de Recherches Forestières (CNR/ISRA), Route des Pères Maristes, BP 2312, Dakar, Sénégal

³ Plant Nutrients and Food Quality Research Group, Plant and Soil Science Section and Copenhagen Plant Science Centre, Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark