

## Article

# Polyloid Advantage? Comparing Salt Stress Responses of Di- and Tetraploid *Acacia senegal* (L.) Willd. Seedlings

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**Abstract:** Polyploidy is often associated with the ability to grow under harsh conditions. The objective of this study was to test whether genome doubling in *Acacia senegal* confers superiority for growth under saline conditions. For this purpose, we assessed the cytotype distribution across ten natural stands in Senegal, representing different soil salinities. We further compared the growth of diploid and tetraploid *A. senegal* seedlings in a shared-pot greenhouse experiment, in which seedling pairs (diploids and polyploids) were irrigated with and without added NaCl. We found polyploids in six stands, and the proportion of polyploids in these stands was significantly and positively correlated with their level of soil salinity. Under experimental conditions, we found that the growth rate of diploid and tetraploid seedlings was equally depressed by salt stress, but the mortality rate for tetraploids was lower than that for diploids (34% versus 54%). In addition, the root-top ratio was higher in tetraploids compared to diploids, which may influence fitness under harsh conditions. Overall, this study provides support for polyploid superiority in *A. senegal* growing in saline soils. The findings of this study may have practical implications for the reforestation of saline soils with polyploid *A. senegal*, and we recommend further studies to elucidate their potential.

**Keywords:** *Senegalia senegal*; survival; growth; polyploidy; salt stress



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## 1. Introduction

Polyploid plants often display novel traits (e.g., increased cell size, changes in physiology, and ecological tolerance to a range of stresses such as cold, drought, and salinity) relative to their parental ancestors, which may enable them to occupy new niches in which their diploid progenitors cannot survive [1–4]. In relation to salt tolerance, it is debated whether polyploidy, in general, is associated with a higher stress tolerance per se, or whether reported cases of polyploids tolerant to salinity represent examples in which poly-ploids have acquired new traits that increase their performance in some specific environments but not in others [5].

Polyploids could be good candidates for the re-vegetation of salt-affected soils as studies on neo-polyploids have shown higher biomass accumulation and less necrosis in polyploids compared to their parental diploids under salinity stress [6–9]. A study by [10] demonstrated increased salt tolerance in naturally occurring tetraploid *Arabidopsis* (*Arabidopsis thaliana*) compared to diploids, and tetraploid rootstock-grafted watermelon (*Citrullus lanatus*) plants were found to be more tolerant to salt stress than their diploid progenitors [11].

The improved performance of polyploids may be due to their better ability to maintain a favorable  $K^+/Na^+$  balance, as observed in *Arabidopsis* [10], or better functioning photosynthetic protective systems, as found in tetraploid *Brassica* compared to its diploid progenitor [7]. Likewise, polyploid *Citrus* cultivars were more tolerant to low salinity levels than diploids [12]; however, the opposite conclusion was reached when the same cultivars were exposed to higher levels of salinity [13], indicating that polyploidy does not always confer better salt tolerance. The question of whether polyploids are generally favored under saline conditions is thus still unresolved, and studies based on trees are scarce.

Recently, the role of polyploidy in adaptation to saline conditions has attracted interest in relation to the socio-economically important African dry zone tree species *Acacia senegal* (L.) Willd. (Fabaceae) (syn. *Senegalia senegal* (L.) Britton). *A. senegal* is reported to naturally grow in salt-affected lands in Senegal, estimated at about 1.7 million hectares [14]. Several studies have highlighted the ability of *A. senegal* to tolerate a high level of salinity [15–18] without considering the potential effect of ploidy level within the species. Thus, understanding and clarifying the basis of observed tolerance in *A. senegal* with respect to genome size is of prime importance, especially in the context of selecting appropriate genotypes for the reforestation of saline zones.

Diploids and polyploids of this species are known to co-occur in Senegal and elsewhere in Africa [19–21], with a high frequency of polyploid cytotypes reported from a saline site in Senegal [22]. This has led us to hypothesize that polyploid plants of *A. senegal* are more tolerant to salinity stress than diploid cytotypes. Here, we test this hypothesis based on the assessment of (i) the distribution of polyploids in relation to soil characteristics (pH and electric conductivity) in natural stands and (ii) the performance of seedlings grown under controlled saline conditions.

## 2. Materials and Methods

### 2.1. Description of Stands with Respect to Salinity and pH

The presence of diploid and polyploid *A. senegal* at 10 sites in Senegal was estimated by [22] based on 30 random trees per site. As we used the site as the experimental unit, soil samples from 0–20 cm depth were collected using a hand auger at 10 randomly selected locations at each site and mixed to constitute one composite soil sample per site. Soil samples were then transported to the ISRA soil lab for analysis. For each composite sample, the electric conductivity (as a proxy for salinity) and pH (assessed in water and KCl solutions) were measured and compared to the estimated frequency of polyploids.

### 2.2. Plant Material for Controlled Salinity Study and Experimental Design

Seeds were collected from one of the natural stands in Diery, in the dry zone of Dahra in Senegal (15°20' N and 15°28' W; annual rainfall < 408 mm). This site has a relatively low level of salinity (Table 1). Seeds from four randomly chosen diploid trees (families: DB17, DB23, DB25, and DB26) and four randomly chosen tetraploid trees (families: DB9, DB10, DB13, and DB14) as assessed in [22] were used for a salt stress experiment.

Seeds were germinated in a climate chamber under a 16 h photoperiod. The temperature in the chamber varied between 28 and 33 °C, while the humidity ranged between 47 and 71%. The ploidy levels of the seedlings were determined by flow cytometric analysis following the method described in [20,21]. At age 2 months, 128 seedlings (64 diploids and 64 tetraploids) were transplanted into pots (13 cm) filled with peat soil (Plugg och Säjord) from Weibulls Horto AB, Sweden. Two seedlings were placed in each pot, one diploid and one tetraploid, in a design in which all diploid families were tested against all tetraploid families. The pairing of plants ensured that diploid and tetraploid seedling pairs were experiencing exactly the same conditions in their common pot, irrespective of size and

water consumption [23]. This makes the two plants in a pot compete for the same resources, thus providing a better impression of their relative performance.

**Table 1.** Site characteristics.

Sites	N	Freq. Diploid (%)	Freq. Polyploid (%)	Latitude	Longitude	Annual Rainfall (mm)	Electric Conductivity ( $\mu\text{S}/\text{cm}$ )	pH Water	pH KCl
Kamb	30	67	33	15°31'32" N	15°26'06" W	400	36.67	6.4	5.7
Diery	30	77	23	15°23'47" N	15°22'54" W	408	14.75	6.1	4.9
Gniby	28	100	0	14°26'11" N	15°38'07" W	555	25.65	6.9	5.8
Velor	30	43	57	14°03'26" N	16°15'25" W	636	802	6.3	5.9
Ngane	25	30	70	14°12'06" N	16°12'09" W	608	1909	6.2	5.8
Pete	30	100	0	16°03'02" N	14°01'04" W	298	24.3	6.6	5.6
Ourouss	30	100	0	15°34'56" N	13°21'54" W	400	19.75	6.7	5.8
Semme	30	93	7	15°11'00" N	12°56'50" W	486	20.99	6.4	5.3
Bakel	30	33	67	14°52'16" N	12°29'20" W	535	19.96	6.0	4.9
Seoudji	30	100	0	14°19'46" N	12°26'43" W	655	27.69	6.2	5.2

Two treatments (control and salt) were applied to the 64 pots, corresponding to 32 pots for the control and 32 pots for the salt treatment (Figure 1).



**Figure 1.** (A) whole experiment: pots with blue labels represent controls; pots with red labels represent plants exposed to salinity. (B) Tetraploid (left) and diploid (right) *Acacia senegal* plants subjected to salt stress.

Pots were randomized and kept in a greenhouse under tropical conditions (minimum temperature of 20 °C and minimum 16 h of daylight for 3 months under well-watered conditions). The experiment began when the seedlings were three months old. The control pots were irrigated with tap water and added standard fertilizer. For the salt stress experiment, the pots were watered with the same fertilizer solution but with increasing concentrations of NaCl (0.05, 0.10, 0.15, and 0.20 mM) to slowly increase the stress level without osmotic shock [23]. Each concentration of salt was applied for 9 days by watering every 3rd day except for the last level, where pots were watered for one month with 0.20 mM of salt, corresponding to a highly saline soil. The pots were irrigated with a large volume (400 mL) of water or salt solution, which leaked from the bottom of all pots. This was done to avoid the buildup of high salt concentrations during salt treatment. During the experiment, the greenhouse temperature on one occasion reached 40 °C, which appeared lethal for some plants and introduced unintended heat stress into the trial.

### 2.3. Measurement of Morphological and Physiological Changes in Response to Salt Stress

Height and diameter were recorded for all individuals at (i) the start of the experiment, (ii) before changing to a higher salt concentration, and (iii) at the end of the experiment.

At the end of the experiment, the number of leaves and survival rate were determined, and the plants were harvested and separated into stems and roots. Survival of plants was evaluated based on leaf loss and the presence/absence of green tissues under the bark (dryness). Tissues were briefly rinsed in water, weighed for fresh weight determination after surplus water was wiped off, and oven-dried at 80 °C for 48 h for assessment of dry weight. Growth and biomass were assessed in surviving plants, considering the values for dead seedlings as missing.

Stem tissues from five control pots and 10 salt-treated pots were used to compare Na<sup>+</sup> and Cl<sup>-</sup> accumulation between diploids and tetraploids. The dried stems were homogenized into a fine powder using a mortar in liquid nitrogen. A 40 mg sample was suspended in 40 mL of water, and the solution was shaken on a horizontal shaking machine at 220 rpm for 30 min to ensure complete dissolution. After this step, all suspensions were filtered through Whatman filter paper, and the concentrations of Na<sup>+</sup> and Cl<sup>-</sup> were measured using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES Perkin Elmer Optima 3000 XL, PerkinElmer Inc., 940 Winter Street, Waltham, MA, USA) and Ion chromatography (Shimadzu Corporation, Kyoto, Japan), respectively.

#### 2.4. Data Analysis

Statistical analyses were performed using R version 3.4.3 (R Core Team 2017).

- Cytotype frequency in relation to site characteristics

We tested for differences in cytotype frequency among the ten sampled natural stands by conducting a chi-square test of homogeneity. Simple regression analyses were performed to examine the links between cytotype frequency (expressed as a percentage) and the following explanatory variables: longitude and latitude (converted to decimal degrees), electric conductivity, rainfall, and pH in water and KCl. Because electric conductivity (EC) (salinity) showed a stochastic rather than a continuous distribution, the data were log-transformed. Additionally, the analysis was supplemented with a one-way ANOVA where the Arcsine transformed frequency of polyploids was modeled as a function of sites with high (EC above 800 µS/cm) or low (EC below 50 µS/cm) salinity.

- Salt stress tolerance evaluation between ploidy levels

Mortality at the end of the experiment was analyzed using a chi-square significance test for the odds ratio (OR) to calculate the odds of a death outcome given exposure versus non-exposure to salt (the estimated likelihood that the diploid group experiences the event (death) compared to the tetraploid group).

Growth and salt accumulation were compared in pots in which both diploid and tetraploid plants survived, corresponding to 30 pots. This was based on a linear model implemented in the *lm* function in R:

$$Y_{ij} = F_d + F_t + Treatment_i + error_{ij}, (i = salt, control; j = (1..32))$$

where  $Y_{ij}$  represents the average or the difference in growth or salinity between the two plants in the same pot (see below), and  $F_d$  and  $F_t$  are the effects of the family of the diploid individual and tetraploid individual in the pot, respectively. First, letting  $Y_{ij}$  represent the average (growth or salt accumulation) of the two plants in a pot, we tested the overall effect of treatment using an F-test. Secondly, to test the effects of ploidy, we let  $Y_{ij}$  represent the difference ( $D_{ij}$ ) between the tetraploid and the diploid plants in each pot, i.e., we used pairwise observations. The hypothesis of no differences between tetraploid and diploid plants in the model corresponds to  $D = 0$ , which was tested using the intercept of the model. Finally, again, letting  $Y_{ij}$  represent the difference ( $D_{ij}$ ) between the tetraploid and diploid plants in each pot, we tested the possible interaction between ploidy level and the

treatment. The hypothesis of a similar response of tetraploid and diploid plants to salt treatment was tested using an *F*-test of the effect of treatment in the model. This approach allowed us to assume that the residuals were independent since each observation was based on a randomized pot. The assumptions of normality and homogeneity of the variance of residuals were checked graphically, and where necessary, a logarithmic transformation was applied to achieve normality. In the case of height, one outlier was deleted due to a typing error.

Bonferroni corrections for multiple tests were not applied to any of the statistical tests. We find this justified since we only performed tests of meaningful biological hypotheses closely linked to the research questions behind the study. However, this means that significant results based on test statistics close to the 5% significance level should be interpreted with caution.

### 3. Results

#### 3.1. Cytotype Occurrence in Relation to Site Characteristics

The variation in ploidy level among sites was statistically correlated with salinity ( $p = 0.02$ ; Tables 1 and 2). The significant relationship between polyploidy and soil salinity was driven by the high frequencies of polyploid trees at the two highly saline sites, Ngane and Velor. This was confirmed by one-way ANOVA comparing the polyploid frequency at these two sites to the other sites ( $p = 0.02$ ). It is important to note that the lower-salinity site Bakel also had a high frequency of polyploidy (see Table 1), and the statistical relationship must, therefore, be interpreted with care.

**Table 2.** Results of simple regressions analyzing the relationship between geographical and edaphic parameters and the frequency of polyploids in % across 10 populations of *Acacia senegal* in Senegal.

Traits	Slope	SE	<i>t</i> -Value	Pr > <i>t</i>
Longitude	0.62	0.66	0.95	0.37
Latitude	0.19	0.9	0.21	0.84
pH water	−71	32	−2.2	0.06
pH KCl	−3	30	−0.1	0.93
log(EC)	32	10	3	<b>0.02</b>
Rainfall	0.13	0.08	1.6	0.15

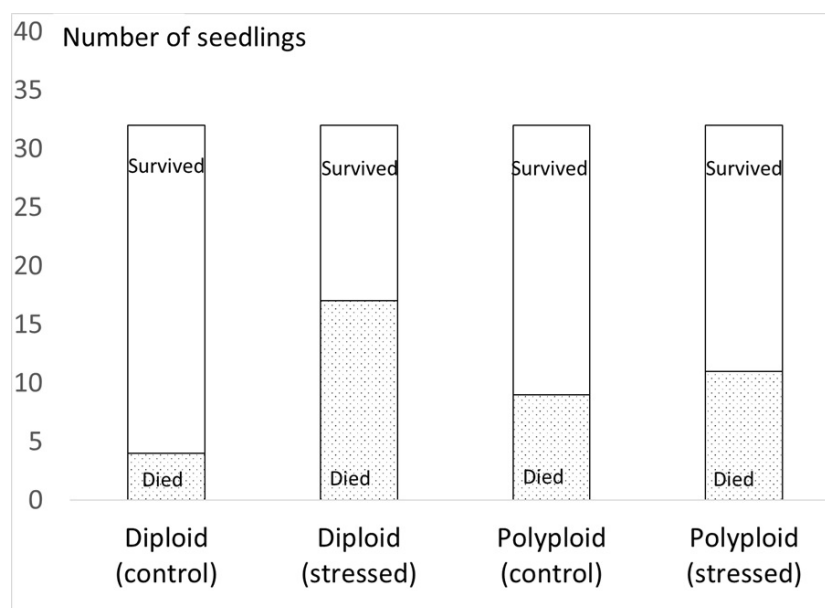
SE: Standard error; Significant value ( $p \leq 0.05$ ) in bold.

No significant relationship was observed between rainfall and cytotype occurrence ( $p = 0.15$ ). The pH (water) was borderline significant ( $p = 0.06$ ); however, the variations in pH were small among sites, and the values were all in the neutral range.

#### 3.2. Performance of Diploid and Tetraploid Seedlings Under Salt Stress

##### 3.2.1. Survival

At the end of the trial, the survival of the control plants was 80%, which differed significantly from the 56% survival of the salt-stressed plants ( $\chi^2(1) = 8.7$ ;  $p = 0.005$ ). Diploid plants had significantly ( $\chi^2(1) = 12.0$ ;  $p < 0.001$ ) larger mortality under salt stress compared to the control treatment (17/32 = 53% versus 4/32 = 13%), while mortality in tetraploid plants was almost unaffected by the salt treatment (11/32 = 34% for salt-stressed plants versus 9/32 = 28% for control) (Figure 2).



**Figure 2.** Variation in survival between diploid and tetraploid *Acacia senegal* seedlings in a salt stress experiment.

When assessing the interaction between cytotypes and treatment, a significant difference was observed ( $p = 0.03$ ), with diploids recording a higher mortality rate under salt stress compared to tetraploids ( $17/32 = 53\%$  versus  $11/32 = 34\%$ ). The analysis of the odds ratio (OR) revealed that diploid seedlings had a 2.74 higher risk of death when grown under saline conditions compared to tetraploid ones (Figure 2).

### 3.2.2. Growth

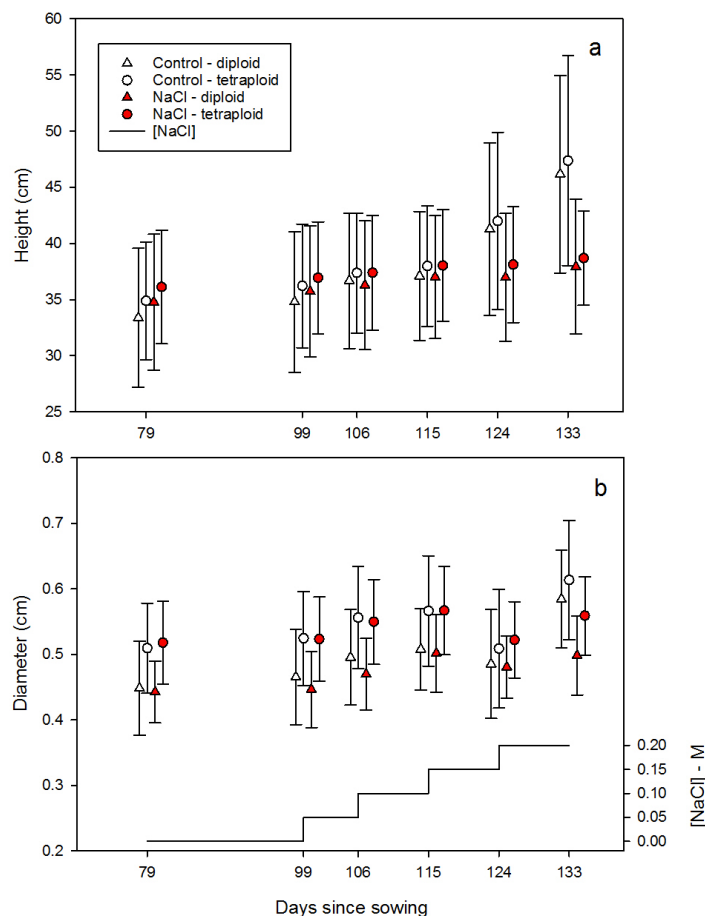
Initially, height and diameter growth were unaffected by salt stress, but at day 124, after exposure to 0.15 mM NaCl, height was significantly lower in salt-stressed plants than in control plants ( $p = 0.0008$ , Figure 3). At the final assessment, after exposure to 0.20 mM NaCl for one month, both height and diameter were smaller in the salt-stressed plants than in the control plants ( $p < 0.0001$  for both). The polyploid seedlings revealed a tendency to grow slightly faster than diploids, but the differences were not significant, and there were no significant interactions between ploidy and treatment, the latter suggesting that the diploid and tetraploid plants reacted in a similar manner to the salt stress for growth traits (Figure 3).

### 3.2.3. Root-Shoot Biomass

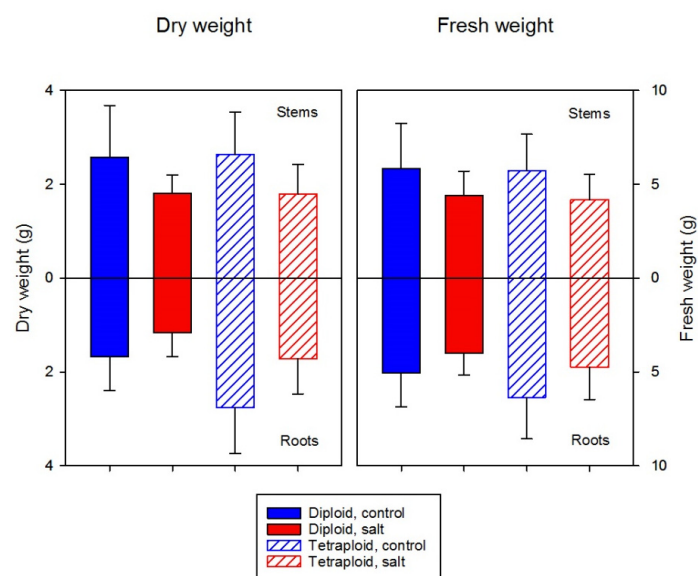
At the time of assessment, several plants had shed their leaves, indicating that comparisons of leaf biomass were not meaningful. However, analyses of stem and root biomass confirmed that seedlings from the unstressed control group were larger than the salt-treated plants, both for fresh and dry weights (Figure 4,  $p < 0.01$  for all).

No interactions were observed between ploidy levels and treatments; however, the fresh and dry weights of the roots were greater in tetraploids than in diploids ( $p = 0.008$  and  $p = 0.004$ , respectively, Figure 4). In addition, the ratio between the fresh weight and dry weight of the roots was significantly higher in diploids than in tetraploids ( $p = 0.03$ ) and was increased in the salt treatments ( $p = 0.01$ ). The ratios between stem and root biomass were not significantly affected by the treatments, but (when calculated on the basis of dry weight) there was a highly significant difference between diploids and tetraploids ( $p < 0.0001$ ), with tetraploids having a larger proportion of the biomass invested in roots compared to diploids (Figure 4). Because such changes could be caused by changed allometry in the

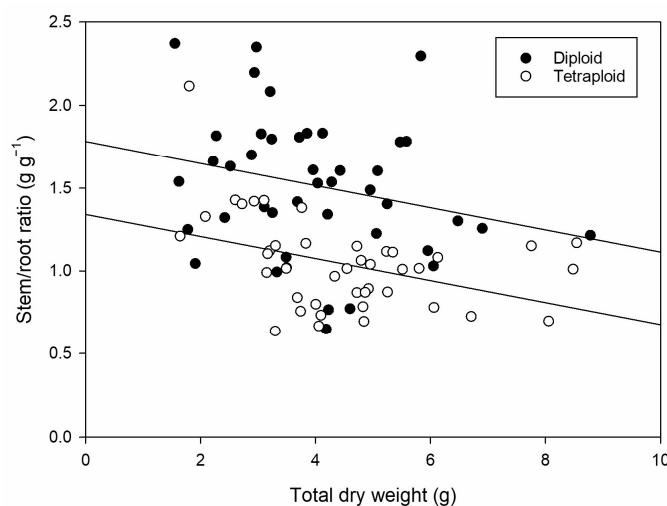
slightly larger tetraploid plants, we plotted the stem/root ratio against the total dry weight of the plants (Figure 5). This shows a decreasing stem/root ratio as plants grow larger, but also a generally higher level of the stem/root ratio in diploids, irrespective of size.



**Figure 3.** Variation in growth in (a) height and (b) diameter between diploid and tetraploid seedlings of *Acacia senegal* under salt stress conditions. The line in the lower part of (b) shows the concentration of NaCl in the irrigation water in the salt stress treatment.



**Figure 4.** Dry and fresh weights of stems (upper) and roots (lower) of diploid and tetraploid *Acacia senegal* in the salt stress experiment. Values represent means  $\pm$  sd (n = 15–28).



**Figure 5.** Differences in stem/root ratios for dry weight between diploid and tetraploid *Acacia senegal* seedlings under salt stress conditions.

#### 3.2.4. Na<sup>+</sup> and Cl<sup>-</sup> Uptake Under Salt Stress

Salt stress treatment resulted in a significant accumulation of salt in the stems of both diploid and tetraploid seedlings ( $p = 0.003$  for Na<sup>+</sup>;  $p = 0.002$  for Cl<sup>-</sup>). The concentration of Na<sup>+</sup> increased from  $5.1 \pm 7.8 \text{ mg g}^{-1}$  to  $39.5 \pm 5.3 \text{ mg g}^{-1}$ , and the concentration of Cl<sup>-</sup> increased from  $9.3 \pm 10.0 \text{ mg g}^{-1}$  to  $58.0 \pm 7.1 \text{ mg g}^{-1}$ . However, there were no significant differences between the two levels of ploidy in their accumulation of salt.

## 4. Discussion

*Acacia senegal* is occasionally found in saline soils in Asia and Africa [21,24] and possesses traits that allows it to tolerate mild salt stress. Plants in our experimental trial, growing under gradually increasing concentrations of salt, accumulated Na<sup>+</sup> and Cl<sup>-</sup> in stems up to levels comparable to levels observed in, e.g., salt-stressed *Citrus* and *Lonicera* cultivars [8,25]. The responses to salt stress observed in the present study were highly significant and typical for *A. senegal*, and included slower growth rates and changed ratios between fresh and dry weights [17,18,24]. From these results, we conclude that our experiment was successful in creating conditions of salt stress.

The experimental design with a diploid and a tetraploid plant growing in the same plot was designed to expose the two plants to the same level of stress, irrespective of the size of the plants and the different transpiration rates that they may have [23]. This design should lead to a strong comparison between plants of the two ploidy levels competing for the same resources. We observed that salt treatment significantly increased mortality in diploids but not in tetraploids, and the odds ratio of 2.7 suggested that diploids had a considerably higher risk of death under salt stress compared to tetraploids. Although care should be taken when extrapolating the survival of seedlings in a greenhouse to field conditions, this supports the hypothesis of better salt adaptation of polyploids and may explain the high frequency of polyploids observed in the Ngane and Velor stands growing on saline soils [22]. Although the effects of salinity may become more severe over time as Na<sup>+</sup> and Cl<sup>-</sup> accumulate in the plant, studies comparing the responses of seedlings with those of mature trees are rare. However, Ref. [26] found that the growth rates of *Populus tremuloides* decreased over time in high-salinity soils compared to sites with low salinity, suggesting that some species may react progressively to salinity. The growth rates of *Picea glauca* under the same conditions were not affected by salinity. Likewise, a screening of *Eucalyptus* for salinity tolerance in Australia under field conditions showed consistent results from age 1 to 4 years, indicating that early results are valid for this genus [27]. In the



case of *A. senegal*, it was previously found that different cytotypes are almost reproductively isolated [21,22]. Thus, given that salt tolerance is stable across the lifetime of *A. senegal*, one may speculate that polyploidization facilitates niche differentiation in this species. In the long term, the higher mortality of diploids in saline soils may lead to the dominance of polyploids in such soils and facilitate the divergent evolution of the two cytotypes. Additional studies are needed to understand and compare the response norms between early life stages and mature trees grown in common gardens.

The changed allometric relationships in tetraploids compared to diploid *A. senegal* that we observed (Figure 4) are likely to influence performance. Previously, we found that tetraploid seedlings grew faster than diploid seedlings under water-stressed conditions in a greenhouse, and in a long-term common garden trial in Senegal, we found superior growth of the tetraploid cytotype [21], supporting the notion of differentiation between diploids and tetraploids in terms of growth. In addition, our results suggest that tetraploids invest more resources in root growth compared to diploids, which could be considered an adaptive mechanism to enhance their survival under stressful conditions.

It has often been reported that drought-tolerant species allocate more resources to root growth than shoot growth for their survival in dry environments [28,29], and it is therefore interesting that we found a relatively larger root growth and better survival in tetraploids compared to diploids (Figure 4). As salt stress is caused by both osmotic stress and the toxicity of  $\text{Na}^+$  and  $\text{Cl}^-$ , plant responses include the accumulation of osmolytes and maintenance of ionic homeostasis [30,31]. Our analysis of  $\text{Na}^+$  and  $\text{Cl}^-$  indicated no differences in ion homeostasis between ploidy levels, but we cannot exclude the possibility that larger root systems in polyploids would increase the uptake of compatible ions, such as  $\text{K}^+$ . Responses to salt stress are controlled by complex interactions among several hormones [30]; however, how this is affected by polyploidy in *A. senegal* is currently unknown.

Polyploids are often associated with increased fitness, usually attributed to the existence of heterosis, allowing polyploids to cope with a broader array of conditions [32]. Polyploids have long been reported to be more frequent in harsh conditions (subarctic regions, high elevations, and xeric environments) and more successful than related diploids in colonizing relatively disturbed areas [1–4]. Polyploidization often leads to the acquisition of novel traits (e.g., increased cell size), genomic restructuring, and changes in gene expression, leading to changes in physiology and ecological tolerance [32–35] that, together with reproductive isolation caused by chromosome doubling, may support adaptation to distinct environmental niches. However, polyploidization often comes at a cost in terms of reduced reproductive success and high levels of apomixis [36], which also seems to be the case for *A. senegal* [20,22], potentially reducing the long-term adaptive potential of this cytotype.

Genetic analysis by [22] revealed a clear separation between diploid and polyploid individuals, but sub-groupings were also found between polyploid genotypes occurring naturally in the saline areas (Ngane and Velor sites) and those used in this experiment (Diery site). Further comparative studies involving polyploid genotypes occurring in saline and non-saline areas are needed to elucidate whether different genetic groups produce different adaptive strategies in response to salt stress.

It would be especially valuable to have studies based on larger plants (e.g., trees that have developed more mature root architecture) growing under conditions close to in situ conditions. However, selection is likely to be especially strong during the very early establishment phase, and studies of seedlings remain highly relevant from an evolutionary perspective.

## 5. Conclusions

Our results revealed that tetraploid *A. senegal* exhibited higher survival rate and root biomass allocation under salinity stress, supporting the hypothesis of better salt tolerance and adaptation in tetraploids. In addition, the observed differences in shoot/root ratios suggest that diploids and tetraploids may have different adaptive strategies to cope with salt exposure. Thus, stands with a higher frequency of polyploid trees could be considered suitable seed sources for future reforestation actions on salt-affected lands. Our results also document the early stage increased survival of tetraploid *A. senegal* under controlled conditions, which is crucial for population establishment and dynamics. However, it remains crucial to verify whether the superiority of tetraploids under salt stress is expressed only at early stages or applies more generally to mature trees. Thus, further studies focusing on comparisons of mature *A. senegal* cytotypes in different habitats or field trials are needed to clarify the genetic basis of salt tolerance mechanisms and confirm long-term adaptive benefits.

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**Data Availability Statement:** The data will be made available in the University of Copenhagen-Electronic Research Data Archive, ERDA.

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## References

1. Te Beest, M.; Le Roux, J.J.; Richardson, D.M.; Brysting, A.K.; Suda, J.; Kubesová, M.; Pysek, P. The more the better? The role of polyploidy in facilitating plant invasions. *Ann. Bot.* **2012**, *109*, 19–45. [[CrossRef](#)]
2. Gunn, B.F.; Murphy, D.J.; Walsh, N.G.; Conran, J.G.; Pires, J.C.; Macfarlane, T.D.; Birch, J.L. Evolution of Lomandroideae: Multiple origins of polyploidy and biome occupancy in Australia. *Mol. Phylogenet. Evol.* **2020**, *149*, 106836. [[CrossRef](#)]
3. Van de Peer, Y.; Ashman, T.L.; Soltis, P.S.; Soltis, D.E. Polyploidy: An evolutionary and ecological force in stressful times. *Plant Cell* **2021**, *33*, 11–26. [[CrossRef](#)] [[PubMed](#)]
4. Ræbild, A.; Ananthawat-Jonsson, K.; Egertsdotter, U.; Immanen, J.; Jensen, A.M.; Koutouleas, A.; Martens, H.J.; Nieminen, K.; Olofsson, J.K.; Röper, A.C.; et al. Polyploidy—A tool in adapting trees to future climate changes? A review of polyploidy in trees. *For. Ecol. Manag.* **2024**, *560*, 121767. [[CrossRef](#)]
5. Buggs, R.J.; Pannell, J.R. Ecological differentiation and diploid superiority across a moving ploidy contact zone. *Evolution* **2007**, *61*, 125–140. [[CrossRef](#)] [[PubMed](#)]
6. Liu, S.; Sumei, C.; Yu, C.; Zhiyong, G.; Dongmei, Y.; Fadi, C. In vitro induced tetraploid of *Dendranthemanankingense* (Nakai) Tzvel.shows an improved level of abiotic stress tolerance. *Sci. Hort.* **2011**, *127*, 411–419. [[CrossRef](#)]
7. Meng, H.; Jiang, S.; Hua, S.; Li, Y.; Guo, W.; Jjiang, L. Comparison Between a Tetraploid Turnip and Its Diploid Progenitor (*Brassica rapa* L.). *Agric. Sci. China* **2011**, *10*, 1671–2927. [[CrossRef](#)]
8. Yan, K.; Wu, C.; Zhang, L.; Chen, X. Contrasting photosynthesis and photoinhibition in tetraploid and its autodiploid honeysuckle (*Lonicera japonica* Thunb.) under salt stress. *Front. Plant Sci.* **2015**, *6*, 227. [[CrossRef](#)]

9. Wang, Z.; Wang, M.; Liu, L.; Meng, F. Physiological and Proteomic Responses of Diploid and Tetraploid Black Locust (*Robinia pseudoacacia* L.) subjected to Salt Stress. *Int. J. Mol. Sci.* **2013**, *14*, 20299–20325. [[CrossRef](#)]
10. Chao, D.-Y.; Dilkes, B.; Luo, H.; Douglas, A.; Yakubova, E.; Lahner, B.; Salt, D.E. Polyploids exhibit higher potassium uptake and salinity tolerance in Arabidopsis. *Science* **2013**, *341*, 658–659. [[CrossRef](#)] [[PubMed](#)]
11. Zhu, H.; Zhao, S.; Lu, X.; He, N.; Gao, L.; Dou, J.; Bie, Z.; Liu, W. Genome duplication improves the resistance of watermelon root to salt stress. *Plant Physiol. Biochem.* **2018**, *133*, 11–21. [[CrossRef](#)] [[PubMed](#)]
12. Saleh, B.; Allario, T.; Dambier, D.; Ollitrault, P.; Morillon, R. Tetraploid citrus rootstocks are more tolerant to salt stress than diploid. *Comptes Rendus Biol.* **2008**, *331*, 703–710. [[CrossRef](#)]
13. Mouhaya, W.; Allario, T.; Brumos, J.; Andrés, F.; Froelicher, Y.; Luro, F.; Talon, M.; Ollitrault, P.; Morillon, R. Sensitivity to high salinity in tetraploid citrus seedlings increases with water availability and correlates with expression of candidate genes. *Funct. Plant Biol.* **2010**, *37*, 674–685. [[CrossRef](#)]
14. Sonneveld, B.G.J.S.; Keyzer, M.A.; Zikhali, P.; Merbis, M.D. *National Land Degradation Assessment Senegal and Review of Global Socio-Economic Parameters in the LADA Data Base*; Land Degradation Assessment Project, LADA Project Paper (Centre for World Food Projects (SOW-VU) Project Papers); Centre for World Food Studies: Amsterdam, The Netherlands, 2010.
15. Pandey, S.; Zhang, W.; Assmann, S.M. Roles of ion channels and transporters in guard cell signal transduction. *FEBS Lett.* **2007**, *581*, 2325–2336. [[CrossRef](#)]
16. Sambou, A.; Ndour, B.; Cheng, S.; Senghor, E. Ligneous species tolerance in acid sulphated and saline soils of sine saloum: Case of rural community of Djilass and Loul Secene. *J. Sustain. Dev.* **2010**, *3*, 174. [[CrossRef](#)]
17. Fall, D.; Bakhoun, N.; Fall, F.; Diouf, F.; Ly, M.O.; Diouf, M.; Gully, D.; Hocher, V.; Diouf, D. Germination, growth and physiological responses of *Senegalia senegal* (L.) Britton, *Vachellia seyal* (Delile) P. Hurter and *Prosopis juliflora* (Swartz) DC to salinity stress in greenhouse conditions. *Afr. J. Biot.* **2016**, *15*, 37.
18. Sarr, M.S.; Seiler, J.R.; Sullivan, J. Growth and physiology of *Senegalia senegal* (L.) Britton seedlings as influenced by seed origin and salinity and fertility treatments. *Forests* **2017**, *8*, 388. [[CrossRef](#)]
19. Odee, D.W.; Wilson, J.; Omondi, S.; Perry, A.; Cavers, S. Rangewide ploidy variation and evolution in *Acacia senegal*: A north-south divide? *AoB Plants* **2015**, *7*, lv011. [[CrossRef](#)] [[PubMed](#)]
20. Diallo, A.M.; Nielsen, L.R.; Hansen, J.K.; Ræbild, A.; Kjær, E.D. Study of quantitative genetics of gum arabic production complicated by variability in ploidy level of *Acacia senegal* (L.) Willd. *Tree Genet. Genomes* **2015**, *11*, 80–92. [[CrossRef](#)]
21. Diallo, A.M.; Nielsen, L.R.; Kjær, E.D.; Petersen, K.K.; Ræbild, A. Polyploid can confer superiority to West African *Acacia senegal* (L.) Willd. trees. *Front. Plant Sci.* **2016**, *7*, 821. [[CrossRef](#)] [[PubMed](#)]
22. Diallo, A.M.; Kjær, E.D.; Ræbild, A.; Nielsen, L.R. Coexistence of diploid and polyploid *Acacia senegal* (L.) Willd. and its implication for interploidy pollination. *New For.* **2022**, *54*, 67–82. [[CrossRef](#)]
23. Verslues, P.E.; Agarwal, M.; Katiyar-Agarwal, S.; Zhu, J.; Zhu, J.K. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *Plant J.* **2006**, *45*, 523–539. [[CrossRef](#)] [[PubMed](#)]
24. Hardikar, S.A.; Pandey, A.N. Growth, water status and nutrient accumulation of seedlings of *Acacia senegal* in response to soil salinity. *Ann. Biol.* **2008**, *30*, 17–28.
25. Ruiz, M.; Pina, J.A.; Alcaide, E.; Morillon, R.; Navarro, L.; Millo, E.P. Behavior of diploid and tetraploid genotypes of ‘carrizo’ citrange under abiotic stress. *Int. Soc. Hort. Sci.* **2016**, *1065*, 1283–1292. [[CrossRef](#)]
26. Lilles, E.B.; Purdy, B.G.; Macdonald, S.E.; Chang, S.X. Growth of aspen and white spruce on naturally saline sites in northern Alberta: Implications for development of boreal forest vegetation on reclaimed saline soils. *Can. J. Soil Sci.* **2012**, *92*, 213–227. [[CrossRef](#)]
27. Zohar, Y.; Di Stefano, J.; Bartle, J. Strategy for screening eucalypts for saline lands. *Agroforest. Syst.* **2010**, *78*, 127–137. [[CrossRef](#)]
28. Aroca, R. *Plant Response to Drought Stress: From Morphological to Molecular Features*; Springer: Berlin/Heidelberg, Germany, 2012; Volume 1, pp. 1–33.
29. Gning, F.; Jourdan, C.; Marone, D.; Ngom, D.; Ræbild, A. Root growth and biomass partitioning of nine juvenile Sahelian agroforestry tree species under drought and irrigation treatments. *Plant Soil* **2025**. [[CrossRef](#)]
30. Zhao, C.; Zhang, H.; Song, C.; Zhu, J.-K.; Shabala, S. Mechanisms of plants responses and adaptation to soil salinity. *Innovation* **2020**, *24*, 100017. [[CrossRef](#)] [[PubMed](#)]
31. Läuchli, A.; Epstein, E. Plant responses to saline and sodic conditions. In *Agricultural Salinity Assessment and Management*; Tanji, K.K., Ed.; ASCE Manuals and Reports on Engineering Practice; ASCE: New York, NY, USA, 2010; pp. 113–137.
32. Levin, D.A. *The Role of Chromosomal Change in Plant Evolution*; Oxford University Press: New York, NY, USA, 2002.
33. Ramsey, J.; Schemske, D.W. Neopolyploidy in flowering plants. *Annu. Rev. Ecol. Syst.* **2002**, *33*, 589–639. [[CrossRef](#)]
34. Paterson, A.H.; Chapman, B.A.; Kissinger, J.C.; Bowers, J.E.; Feltus, F.A.; Estill, J.C. Many gene and domain families have convergent fates following independent whole-genome duplication events in *Arabidopsis*, *Oryza*, *Saccharomyces* and *Tetraodon*. *Trends. Genet.* **2006**, *22*, 597–602. [[CrossRef](#)] [[PubMed](#)]

35. Alix, K.; Gérard, P.R.; Schwarzacher, T.; Heslop-Harrison, J.S. Polyploidy and interspecific hybridization: Partners for adaptation, speciation and evolution in plants. *Ann. Bot.* **2017**, *120*, 183–194. [[CrossRef](#)] [[PubMed](#)]
36. Hojsgaard, D.; Hörandl, E. The rise of apomixis in natural plant populations. *Front. Plant Sci.* **2019**, *10*, 358. [[CrossRef](#)] [[PubMed](#)]

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