The effect of facilitation on two *Brachypodium distachyon* cytotypes
Contents
Abstract ........................................................................................................................................ 3
Introduction .................................................................................................................................. 4
Methods ......................................................................................................................................... 6
Results ........................................................................................................................................... 9
Discussion ..................................................................................................................................... 19
References ...................................................................................................................................... 21
Abstract
Over the years the importance of facilitation between plants has been recognized more and more. Facilitation has been shown to alleviate the environmental stress experienced by their beneficiaries. Since climate change is expected to increase the environmental stress for many species, facilitation could play an important role in diminishing the impact of climate change.

However the long term effects of facilitation are not know. Studies have shown that facilitation can preserve lineages maladapted to their environment, but can it also lead to evolutionary adaptations? It is theorized that environmental conditions can affect the haploid ratio in plants. Facilitation can thus affect different cytotypes differently, leading to differences between facilitated and non-facilitated populations (especially mixed populations). On the long term these population differences can lead to genetic differences between the populations. Using the annual beneficiary grass *Brachypodium distachyon* as a model system, I tested the following hypothesis: Facilitation leads to a lower tetraploid/diploid ratio of *Brachypodium distachyon*.

To test this hypothesis, *Brachypodium* was grown in the lab under wet and dry conditions to simulate facilitation. The results of this experiment show that less water significantly lowers the aboveground biomass for tetraploid plants, but not for diploid plants. In both wet and dry conditions the tetraploids outperformed the diploids. So, the tetraploids and not the diploids were most affected by the dry treatment. Tetraploids flowered earlier but had a reduced number of flowers and seeds compared to the wet treatment. Diploids flowered later, but had the same number of flowers and seeds compared to the wet treatment. These results show that each cytotype has a different strategy in dealing with drought.

Facilitation has differential effects on the two cytotypes, leading to different ratios in mixed populations. Future research should focus on multiple generations to understand the evolutionary consequences of these population differences and how different traits may evolve over time.
Introduction

Plants interact in many different ways with their environment and with each other. They interact in a negative way and compete for resources (light, nutrients and water), space and pollinators (i.e. competition). But they can also interact in a positive way (i.e. facilitation) by protecting each other from the impacts of herbivores, other competitors or climatic extremes (Brooker et al. 2007). Over the years plant-plant interactions have received more and more attention and our understanding of them has grown (Brooker et al. 2007). Especially the importance of facilitation, an interaction in which the presence of one species alters the environment in a way that enhances growth, survival or reproduction of a second, neighbouring species (Bronstein 2009), has been recognised more and more (Brooker et al. 2007; Bronstein 2009). However, the long term effects (evolutionary responses) of facilitation among plants themselves are far less studied, but could provide us with a greater insight into processes that organize communities (Thorpe et al. 2011). Especially in light of the predicted global environmental changes, which will increase the stress level for many species, there is a clear need to better understand the long term effects of facilitative interactions, as facilitation can alleviate the increased stress induced by environmental change. If facilitation could lead to evolutionary adaptations, facilitation may play an important role in diminishing the impact of global environmental change on plant communities (Soliveres and Maestre 2014). Current research shows that facilitation can preserve lineages less adapted to changing environmental conditions, by working as an ecological time machine for less adapted lineages and pull, as it were, these lineages through time over millions of years (Soliveres and Maestre 2014; Valiente-banuet, Rumebe, and Verdu 2006). Whether this preservation due to facilitation also leads to facilitation induced evolutionary changes is not known and will be the topic of this proposal.

An interesting phenomenon is that some plant species exhibit variation in polyploidy levels, i.e. the acquisition of more than two sets of chromosomes (te Beest et al. 2012). It is hypothesized that these different cytotypes can be an adaptive response to different environmental conditions. Since facilitation can change the way plants experience their direct environment, it may also affect polyploidy levels in plant communities. Current research indeed indicates that polyploidy can be an adaptation to different environmental conditions. Research on polyploidy on many different species indicated that polyploidy may lead to an increase in phenotypic plasticity, competitiveness, drought tolerance, colonization ability and range (te Beest et al. 2012). Centaurea stoebe, for example, is a species that is much studied when it comes to polyploidy (Mráz, Tarbush, and Müller-Schärer 2014; Mráz et al. 2012; Hahn, van Kleunen, and Müller-Schärer 2012; Otisková et al. 2014). The main conclusion that came from all these different researches, was that tetraploids were mostly found in disturbed (man-made) areas and the diploid type in the more natural areas. The tetraploid also showed an increase in geographical distribution and phenotypic plasticity. Mráz et al. (2012) also found that tetraploids were more drought tolerant than the diploids, but only for some populations not all. Manzaneda et al. (2012) also claimed to have found an increase in drought tolerance for Brachypodium distachyon. Although they concluded that this was indeed the case, their results were not clear and showed minimal association between cytotype segregation and environmental aridity. They did show an increase in drought related traits in the tetraploids compared to the diploids. The difference between the two studies is that Manzaneda et al. used a combination of precipitation and temperature as a measure for drought, whereas Mráz et al. (2012) used ground moisture as a measure for drought. This ground moisture content may be a far better measure for drought, because water availability is not only determined by precipitation and temperature (i.e. evaporation rate) but also by soil type. Some soils are better in retaining water than others (Hao et al. 2012). Mráz et al. (2014) tested this increased tolerance to drought in the lab, where they subjected different geocytotypes of Centaurea stoebe to three different watering treatments. They found that the tetraploidy was slightly more drought tolerant than the diploids. But the
effects were most likely blurred by population and individual differences, so the effect of polyploidy could be bigger than found in their experiment.

Currently experimental research on facilitation induced evolutionary adaptations is non-existent. But also research on how facilitation may affect population dynamics is non-existent. It is however important to know these population effects of facilitation, because these populations are at the base of the evolutionary adaptations. Facilitation can affect different populations in different ways, for example when these populations consists of different plant cytotypes. As mentioned before some cytotypes may be more benefitted by facilitation than others, so populations consisting of different mixtures of these cytotypes may be affected differently. Facilitation will affect population dynamics of each population differently and will thus lead to different evolutionary adaptations. So, there is a clear need for experiments testing the population and evolutionary consequences of facilitation. A better understanding of facilitative interactions and their long term effect will help us to better deal with restoration and conservation challenges imposed by global environment change. Also current research shows that there is an indication that higher ploidy leads to higher drought tolerance, but the results thusfar show only minimal association. So in this research project I will look at the effect of drought on polyploidy and the effect facilitation might have on the evolution of polyploidy. In this project I will use the annual beneficiary grass *Brachypodium distachyon* from Spanish semi-arid *Stipa tenacissima* steppes as a model system, in which *Brachypodium* is facilitated by *Stipa*.

**I expect that:**

Facilitation, by offering wetter environmental conditions, leads to a lower tetraploid/diploid ratio of *Brachypodium distachyon* in mixed populations, because the diploids are less adapted to drought.
Methods

Study system
This experiment was carried out using the annual beneficiary grass *Brachypodium distachyon* from Spanish semi-arid *Stipa tenacissima* steppes as model system (fig. 1). These semi-arid *Stipa* steppes cover a large area in the Mediterranean, from Southern Spain to North Africa.

*Brachypodium* is a temperate annual small grass (10–20 cm). It grows in a wide variety of habitats under different climatic and ecological conditions although it is mostly found either in altered habitats such as roadsides or abandoned fields, and in natural xerophytic meadows and forest edges (Manzaneda et al. 2012). *B. distachyon* has a short life-cycle, although its life span varies remarkably among genotypes (seed to seed cycle time ranges from 3 to 20 wk) (Manzaneda et al. 2012; Brkljacic et al. 2011). *B. distachyon* is self-compatible and seed production occurs during summer (June–August), but the species may perform either as a winter annual (germination occurs between September and November), or as a spring annual (germination occurs in March–April) (Manzaneda et al. 2012). An interesting phenomenon of *Brachypodium* is that it exhibits variation in polyploidy levels. It is hypothesized that these different cytotypes can be an adaptive response to different aridity conditions (Manzaneda et al. 2012). These cytotypes can occur in populations of one cytotype, but also in mixed populations of multiple cytotypes (Manzaneda et al. 2012). Field experiments have shown that *Brachypodium* occurs in association with *Stipa tenacissima*. These studies have shown that *Stipa* can facilitate a wide range of less stress-tolerant species (Saiz and Alados 2011; Maestre and Cortina 2004). *Brachypodium distachyon* is one of these facilitated species (Saiz and Alados 2011). One of the facilitative aspects of *Stipa*, is that it creates wetter surroundings for its beneficiary species. Especially in mixed populations (multiple cytotypes) facilitation can be an important factor in determining population dynamics.

Experimental set-up
To test the hypothesis that facilitation leads to a lower tetraploid/diploid ratio of *Brachypodium distachyon* in mixed populations, diploid and tetraploid plants of *Brachypodium distachyon* were grown in pots under two different watering treatments (high and low water availability). The pots consisted of a mixed population of diploid and tetraploid plants (5/5 ratio). The different watering treatments simulate the facilitative effect of *Stipa tenacissima*. High water treatment represents facilitation and low water treatment represents no facilitation.
Pre-treatment sowing and growing

In this experiment seeds were used from the diploid *Brachypodium distachyon* line Adi-1 (w639234) and from the tetraploid *Brachypodium distachyon* line Adi-P1 (w639252). The seeds were gifted by Washington State University. To determine if there was a difference in seed size between diploid and tetraploid seeds, the seeds were individually weighed. The seeds were then planted in individual pots, containing a mix of 69% sand (metselzand) and 31% organic potting soil (lentse potgrond) (roughly the same mixture as found under natural conditions (Rey et al. 2011)). The pots were covered in a plastic wrap and placed in a dark room at 4 °C for 6 days, to allow for vernalisation.

After 6 days of vernalisation, the plants were placed in a greenhouse (day 0) under long-day (16 hours light/8 hours dark) conditions at 23 °C (Manzaneda et al. 2012; Draper et al. 2001). The pots were placed in trays containing water, to keep the soil moist (fig. 2). After germination the plastic cover was removed. Germination was scored to see if there was a difference between the diploid and tetraploid line. After 18 days 40 diploid and 40 tetraploid plants were transplanted to the treatment pots. Only plants that germinated after 2 or 3 days were used in the treatment pots, to control for benefits of early germination.

![Fig. 2: Pre-treatment growing of Brachypodium distachyon](image)

Treatment

The treatment consists of 8 40L pots, 4 with a wet treatment and 4 with a dry treatment. Each pot contains a mixed population of diploid and tetraploid individuals (5/5 ratio), placed in a circle alternating diploid and tetraploid plants (fig. 3). This was done to make sure that they were evenly distributed. 5 diploid and 5 tetraploid plants of roughly the same sizes were placed each 40L pot. The treatment pots were watered (10L of water per pot) 4 days prior to translocation to make sure that the plants were not planted in completely dry soil. The plants were then left to grow until flowering. The pots were rotated every few weeks to control for location effects. The wet treatment was initially given 2.5L of water a week and the dry treatment nothing. But halfway the experiment (around day 53) both the dry and wet treatment suffered greatly from drought stress and to avoid that all plants died, the water regime was increased to two times 2.5L a week for the wet treatment and one time 2.5L a week for the dry treatment.

During the experiment plant length (to nearest mm), leaf width (to nearest 0.5 mm) and number of sprouts were measured to obtain an estimate of the plant growth and above ground biomass. Length and number of sprouts were measured for all plants. Leaf width was measured by taking the average of 3 random leafs of each plant. The soil water content in each pot was measured almost every day to evaluate the effectiveness of the two treatments. The soil water content was measured with a WET sensor at 3 depths (6 cm from the bottom (layer C), 12 cm from the bottom (layer B) and 18 cm from the bottom (layer A)) at 4 opposing sides of the pot, so 12 measurements per pot. We also used a porometer to test for drought stress. A porometer measures the openness of the stomata, the lower the value
the more open the stomata. The porometer data can thus be used to indicate drought stress, as drought will cause the stomate to close.

Flowering and harvesting
After most of the plants had flowered, the flowers and the rest of the aboveground biomass were harvested. The flowers were harvested at day 119 and the rest of the aboveground biomass was harvested at day 122. In comparison a normal growing season for Brachypodium in the Mediterranean last about 3 months (June-August), so under natural conditions our plants would have dried out. Per plant we determined the following: Flowering time of first flower (days), number of flowering stems, number of spikelets (fig. 4), number of florets (fig. 4), reproductive biomass (cg) (flowers and seeds) both fresh and dry and aboveground biomass (g) both fresh and dry.

Statistics
Each treatment was replicated 4 times with 10 Brachypodium individuals per treatment, 5 diploids and 5 tetraploids. A T-test was used to test if there is a significant difference (P<0.05) in seed size and germination time between the diploid and tetraploid line. For plant
length, leaf width, number of sprouts, flowering time, number of flowering stems, number of spikelets, number of florets and the biomass measurements an ANOVA was used to test for effects of treatment, cytotype and their interactions. If there was a significant difference (P<0.05) and interaction (between treatment and cytotype) between the four groups, WD (wet treatment diploid plants), WT (wet treatment tetraploid plants) DD (dry treatment diploid plants) and DT (dry treatment tetraploid plants). If significant it was followed by a Tukey’s test. Also a Kaplan-Meier survival analysis was done on the flowering time data, to test for significant differences between the four groups over the length of the experiment.

Results

Pre-treatment sowing and growing

Figure 5 shows the weight distribution of the diploid and tetraploid Brachypodium seeds. The diploid seeds with an average weight of 5.3 ± 2.2 mg differed significantly (t-test: t-value = 27.692; P<0.001) from the tetraploid seeds with an average weight of 9.3 ± 2.1 mg. After germination one of the seeds actually turned out to be two seeds. In the analysis the average weight of these two were used for both seeds. Of the diploid seeds 72.1% germinated and of the tetraploid seeds 78.6% germinated. Figure 6 shows the distribution of germination time of the diploid and tetraploid Brachypodium plants. The diploid plants had an average germination time of 3.3 ± 3.6 days and the tetraploid plants had an average germination time of 2.4 ± 0.7 days. There was no significant difference (t-test: t-value = 1.699; P>0.05).

![Seed distribution of the diploid and tetraploid Brachypodium seeds](image)

**Fig. 5:** Distribution of the diploid (blue) and tetraploid (red) Brachypodium seeds according to weight (mg).
Treatment (drought & cytotype)

Figure 7a and b show the length of diploid and tetraploid plants and water content of the soil during the experiment, for the wet and dry treatments respectively. Fig. 7a (wet treatment) shows that tetraploids started out taller than the diploids, but the diploids caught up and even grew taller than the tetraploids before being surpassed by the tetraploids at the end. Figure 7b (dry treatment) shows that the tetraploids started out taller, but the diploids caught up and surpassed the tetraploids. The graphs (7a and b) also show that the plants have a stepped grow curve, that the increase in height is the most 2 to 3 days after watering and that DT stopped growing earlier than DD, WD and WT. At the end of this study (122 days) there was a significant difference between the groups (ANOVA: F<sub>3, 79</sub> = 48.055; P < 0.001). A tukey test showed that the average length of WD (243.2 ± 15.24 mm) did not differ significantly from WT (250.5 ± 21.76 mm). DD (222.7 ± 20.48 mm) differed significantly from DT (187.6 ± 14.06 mm), WD and DD differed significantly and WT and DT differed significantly. After 42 days the leaves of DT started welching. The leaves of DD started welching after 46 days and WT after 53 days. The graphs (7a and b) show that at this point the soil moisture content was also at its lowest.
Figure 8 shows the average leaf width during the experiment for the four groups, WD, WT, DD and DT. There was a significant difference between the groups (ANOVA: $F_{3, 79} = 171.873; P < 0.001$). A tukey test showed that the leaf width of WD ($5.38 \pm 0.22 \text{ mm}$) and DD ($4.75 \pm 0.34 \text{ mm}$) differed significantly from WT ($6.63 \pm 0.23 \text{ mm}$) and DT ($5.53 \pm 0.26 \text{ mm}$) across the whole experiment except on day 50 when there was no significant difference between DD and DT, there was however a significant difference between WD and WT. WD and DD did not differ significantly initially, but started to differ significantly from day 36.
onwards. WT and DT also did not differ significantly initially, but started to differ significantly from day 29 onwards.

Figure 9 shows the number of sprouts per plant during the experiment for the four groups, WD, WT, DD and DT. There was a significant difference between the groups (ANOVA: \( F_{3,79} = 88.143; P < 0.001 \)). A tukey test showed that the number of sprouts per plant of WD (36.3 ± 7.31) and WT (78.4 ± 13.13) differed significantly from DD (30.6 ± 6.80) and DT (58.3 ± 12.75) across the whole experiment. WD and DD also differed significantly across the whole experiment. WT and DT did not differ significantly initially, but started to differ significantly from day 43 onwards.

**Fig. 8:** Average leaf width (mm) during the experiment for Diploid wet (solid blue), Diploid dry (dotted blue), Tetraploid wet (solid red) and Tetraploid dry (dotted red)
Flowering and harvesting

Figure 10 shows the average flowering time of each of the four groups. There was a significant interaction between treatment and cyto type (ANOVA: $F_{1,53} = 5.031; P < 0.05$). Fig 10 also shows the differences between the groups (as tested by a tukey test). Only DD (118.5 ± 2.2 days) and DT (106.1 ± 13.6 days) differed significantly. WD (108.3 ± 7.5 days) and WT (108.8 ± 11.9 days) did not differ.

Fig. 9: number of sprouts per plant during the experiment for Diploid wet (solid blue), Diploid dry (dotted blue), Tetraploid wet (solid red) and Tetraploid dry (dotted red)

Fig. 10: Average flowering time and error bars of WD (light blue), WT (light red), DD (dark blue) and DT (dark red). The letters above the bar represent significant differences.
Figure 11 shows the average number of flowering stems of each of the four groups. There was a significant interaction between treatment and cytotype (ANOVA: F1, 53 = 14.46; P < 0.001). Fig 11 also shows the differences between the groups (as tested by a tukey test). WT (26.4 ± 12.7) differed significantly from the other groups and the other groups. WD (3.9 ± 2.4), DD (2.5 ± 1.7) and DT (7.6 ± 6.3) did not differ among each other.

![Figure 11](image1.png)

**Fig. 11:** Average number of flowering stems and error bars of WD (light blue), WT (light red), DD (dark blue) and DT (dark red). The letters above the bar represent significant differences.

Figure 12 shows the average number of spikelets of each of the four groups. There was a significant interaction between treatment and cytotype (ANOVA: F1, 53 = 14.73; P < 0.001). Fig 12 also shows the differences between the groups (as tested by a tukey test). WT (58.6 ± 27.7) differed significantly from the other groups and the other groups. WD (9.6 ± 7.7), DD (7 ± 4.6) and DT (17 ± 14.3) did not differ among each other.

![Figure 12](image2.png)
Figure 13 shows the average number of florets of each of the four groups. There was a significant interaction between treatment and cytotype (ANOVA: $F_{1, 53} = 12.29; P < 0.001$). Fig 13 also shows the differences between the groups (as tested by a tukey test). WT ($273.3 \pm 140.1$) differed significantly from the other groups and the other groups. WD ($29 \pm 21.8$), DD ($21 \pm 13.6$) and DT ($86.3 \pm 77.0$) did not differ among each other.

Figure 14a-b show the average fresh and dry weight of the reproductive biomass (RB) of each of the four groups. There was a significant interaction between treatment and cytotype (dry ANOVA: $F_{1, 53} = 5.304; P < 0.05$) (fresh ANOVA: $F_{1, 53} = 9.495; P < 0.01$). Fig 14a-b also show the differences between the groups (as tested by a tukey test). WT (fresh $1.574 \pm 0.95$ cg, dry $0.633 \pm 0.41$ cg) differed significantly from the other groups and the other groups. WD (fresh $0.074 \pm 0.059$ cg, dry $0.037 \pm 0.034$ cg), DD (fresh $0.045 \pm 0.029$ cg, dry $0.019 \pm 0.013$ cg) and DT (fresh $0.467 \pm 0.56$ cg, dry $0.232 \pm 0.34$ cg) did not differ among each other.
Figure 15a-b show the average fresh and dry weight of the aboveground biomass (AGB) of each of the four groups. There was a significant interaction between treatment and cyto type (dry ANOVA: \( F_{1, 76} = 32.32; P < 0.001 \) ) (fresh ANOVA: \( F_{1, 76} = 24.29; P < 0.001 \) ). 14a-b also show the differences between the groups (as tested by a tukey test). WT (fresh 30.8 ± 9.6 g, dry 14.5 ± 3.5 g) and DT (fresh 15.19 ± 4.3 g, dry 6.84 ± 1.9 g) differed significantly from the other groups and each other. WD (fresh 8.8 ± 5.0 g, dry 4.8 ± 2.0 g) and DD (fresh 6.3 ± 2.6 g, dry 3.0 ± 1.1 g) did not differ from each other.
Figure 16 shows the Kaplan-Meier (KM) survival analysis for the flowering time data. Statistical analysis shows that there is a significant difference between the groups (Chisq = 13 on 3 degrees of freedom, P < 0.01).

**Fig. 15a:** Average fresh weight (g) of the aboveground biomass and error bars of WD (light blue), WT (light red), DD (dark blue) and DT (dark red). The letters above the bar represent significant differences.

**Fig. 15b:** Average dry weight (g) of the aboveground biomass and error bars of WD (light blue), WT (light red), DD (dark blue) and DT (dark red). The letters above the bar represent significant differences.
Figure 17 shows the porometer data for the different groups. A total of three measurements were done but no statistics was done because there were doubts over the reliability of the data. To perform a good measurement a leaf has to cover a small area in order to give a good reading, but during the first measurement, the leaves of the diploid plant were too small to cover the entire area, the actual values for the diploids may in fact thus be lower. There was no problem with the tetraploid leaves. In the third measurement we could only get a reading for the wet treatment. We were unable to get a reading from the dry treatment, most likely because the stomata were almost closed.

![Figure 16: Survival analysis of flowering time for WD (green), WT (blue), DD (black) and DT (red).](image)

![Figure 17: Porometer data for WD (blue solid), WT (red solid), DD (blue dotted) and DT (red dotted).](image)
Discussion

In this experiment we tried to understand the effect of facilitation on different cytotypes (diploid and tetraploid) of *Brachypodium distachyon*. We indeed found different effects for the different cytotypes, mainly in when they flower and how much seed they produce. Facilitation thus has differential effects on the two cytotypes, leading to different ratios in mixed populations.

Our data shows that the tetraploid plants start out in the beginning with an advantage over the diploid plants. The tetraploid seeds were almost twice as large as the diploid seeds. Literature indeed states that polyploids usually have bigger seeds (te Beest et al. 2012). This means that they also have almost twice as many resources to start with, which can give them an advantage in the earlier growing stages (Vaughton and Ramsey 1998; Turnbull, Rees, and Crawley 1999). The reason for these bigger tetraploid seeds could be that tetraploids have more genetic material (Comai 2005) and thus bigger seeds. The tetraploids also had a higher germination success than the diploids, 78.6% versus 72.1%. The differences are not big, but can be just enough to give tetraploids an edge early on. This difference in germination time is not affected by seed mass (Vaughton and Ramsey 1998).

The treatment effect on length, leaf width and number of sprouts was strongest in the tetraploid. This is not what we expected, because literature suggested that polyploids were in fact better able to handle drought conditions (te Beest et al. 2012; Mráz, Tarbush, and Müller-Schärer 2014; Mráz et al. 2012; Hahn, van Kleunen, and Müller-Schärer 2012; Otisková et al. 2014). But although the tetraploids were more affected they had a clear size advantage (both in length, leaf width and number of sprouts) over the diploids in both treatments. However, the growth rate for the diploid and tetraploid plants is about the same initially, suggesting that the bigger size of the tetraploids may be an effect of larger seed mass and earlier germination (Turnbull, Rees, and Crawley 1999; Vaughton and Ramsey 1998). Our results suggest that the tetraploids are in fact not better adapted than the diploids which contradicts the study done by Manzaneda et al 2012. They found that the tetraploids coped better with arid conditions. This can be explained by that, although the tetraploids seemed more affected by the dry treatment than the diploids, they were still bigger than the diploids. In fact the tetraploids outperformed the diploids in all treatments, suggesting that the tetraploids have an overall advantage over the diploids and not only in dry conditions as literature suggested. Most studies studying drought and polyploidy compare the fitness of different cytotypes under one drought condition, which results in the conclusion that polyploids are better drought adapted. But as our study shows, polyploids may perform better under all conditions. This suggest that the result found in previous studies may not be an effect of better drought tolerance in polyploids but of better overall performance in polyploids. Indeed studies have shown that polyploids are in general bigger (te Beest et al. 2012).

In this experiment we started out with a water regime of 2.5L a week for the wet treatment and no water for the dry treatment. But after 42 days the leafs of DT plants started welching, followed by DD at 46 days and WT at 53 days. So both treatments, even the wet, were starting to show serious signs of drought. In order to save the plants, we increased the water regime to two times 2.5L a week for the wet treatment and one time 2.5L a week for the dry treatment. We believe this drought stress was not due to our water regime but due to the amount of water we gave the treatment pots before transplanting the plants. Before transplanting, each of the treatment pots were give 10L of water each to water and compact the soil. We expected that in the greenhouse this bare soil would dry up rather quickly, which was the case for the upper layer, but not for the bottom. This high soil moisture content allowed the plants to acquire more biomass, than they would normally have under our water regimes. Because of the water regimes inability to maintain those initially high soil moisture content, the plants could not maintain the fast biomass they had acquired and started to dry out. This could also explain why the tetraploids, contrary to current knowledge, were more affected than the diploids. The tetraploids performed better than the diploids in both treatments and acquired more biomass, which means it also had to maintain more biomass and thus needed more water. In future experiments it would be best to use 2.5L of water...
(same as treatment) instead of 10L to water the pots and instead of not watering the dry
treatment at all, water it every two or three weeks with 2.5L. This probably will not changes
our results, but will probably reduce the drought effect on both cytotypes.

Our data shows that the tetraploids are more affected by the dry treatment than the
diploids and that both cytotypes have completely different strategies in dealing with drought.
In the wet treatment both diploids and tetraploids flowered at the same time, but in the dry
treatment the diploids flowered significant later than the tetraploids. So the diploids in the dry
treatment seem to flower later than the diploids in the wet treatment and the tetraploids in the
dry treatment seem to flower earlier than the tetraploids in the wet treatment, which is not
uncommon under drought stress (Fox 1990). The diploids had the same amount of flowering
stems, spikeletes, florets and reproductive biomass in both treatments, but the tetraploids
had a significant lower amount in the dry treatment than in the wet treatment. The tetraploids
flower earlier but also reduce their reproductive capability and the diploids flower later but
keep the same reproductive capability. The tetraploids can still have an advantage because
they flower earlier, the diploids will still have to live long enough to be able to make flowers.
Although the tetraploids had lower reproductive capability in the dry compared to the wet,
they still had the same reproductive capability as the diploids, again suggesting that the
tetraploids overall perform better than the diploids.

Although we were able to grow only one generation of Brachypodium we can still
make some predictions for the next generation based on the number of florets and
germination success. The average number of florets per plant were 29 for WD, 273 for WT,
21 for DD and 86 for DT. Assuming each floret will develop and contain one seed and the
germination success will be roughly the same (72.1% for the diploids and 78.6% for the
tetraploids) the next generation will thus consist of 20.9 (29*0.721) WD plants, 214.6
(273*0.786) WT plants, 15.1 (21*0.721) DD plants and 67.6 (86*0.786) DT plants. The
tetraploid/diploid ratio in the wet treatment will thus be 214.6/20.9=10.3 and in the dry
treatment 67.6/15.1=4.5. So contrary to what we expected facilitation (wet treatment) leads to
a higher tetraploid/diploid ratio than with no facilitation (dry treatment). This could be
explained by the fact that the tetraploids and not the diploids were the ones that were most
affected by the dry treatment. The ratio’s also show that although we started with a 1/1 ratio
in both treatments, the tetraploid/diploid ratio increases extensively in both treatments. The
next generation will thus consist mainly of tetraploids. This also suggests that in later
generations the tetraploids could have entirely replaced the diploids. It would be interesting
to see whether this also applies to other species with multiple cytotypes.

Our results show that tetraploids and diploids have different strategies in dealing with
drought and that although tetraploids outperformed diploids in both treatments they were
most affected by it. The predictions we made also shows that future generations may consist
entirely of tetraploids and that, unlike we expected, facilitation may in fact favour tetraploids
and not diploids. However our study only followed one generation of Brachypodium. Future
studies should aim to study multiple generations. Only with the study of multiple generations
would we be able to understand the effects of facilitation on these different cytotypes. Also in
this experiment we used two different water regimes to simulate facilitation (wet) and no
facilitation (dry). However facilitation could have more effects than only increased ground
moisture content. It could provide shade or grazing protection. Future studies should
therefore also aim to include an actual facilitator in their experiments. Our study provides us
with new insights in how different cytotypes react to different water regimes and how this
could affect next generations, but it also show that much is yet unknown and that more
research is needed to better understand the mechanisms behind it.
References


Mráz, Patrik, Stanislav Španiel, Andreas Keller, Gillianne Bowmann, Alexandre Farkas, Barbora Šingliarová, Rudolf P Rohr, Olivier Broennimann, and Heinz Müller-Schärer.


