**TITLE : Durum wheat plantlet behaviour toward neighbors’ genotype under different hydric conditions and varieties mixture**

**Abstract**

Variety mixture is a very promising way to improve the performance of crops according to ecological theories of facilitation and niche complementarity. Yet, the results of mixtures compared to monoculture are often very variable, and not well understood.

In this article we look if among several varieties of durum wheat, some will have an impact on the neighbor early development, according to its genotype and the hydric conditions

We put 2 plants, of the same variety or not, in a pot under two different watering conditions. We tested 15 varieties, half that owned a nanism gene and the other that did not. As an indicator of productivity we chose to measure the dry biomass at 21 days of growth. This factor could not be linked to the neighbors' alikeness. We did not find that the watering condition had an impact either. We have some results at the genotypic scale. One of the genotypes grows differently regarding its neighbor. Deeper experiments focused on this genotype could be very interesting to understand the mechanism behind its functioning. The research among a more important number of varieties is necessary to explore and understand what really makes a plant sensitive or not to its neighbor.

**Keywords**

Plant ecological interaction, Durum wheat, crop/variety mix, intraspecific diversity, overyielding, EPO collection, water stress.

**Are there intra-varietal interactions depending on hydric conditions within durum wheat (triticum durum) ?**

**Introduction**

The selection and cultivation of plant species by man has led over the centuries to the domestication of these plants and their standardization according to the characteristics that interest them most. The result is a very low intra-varietal diversity of elite lines and this homogeneity makes them vulnerable and dependent on inputs and pesticides to maintain their high yield. Varietal mixtures, that is, the cultivation of different genotypes within a field, have received renewed interest in recent years with the growing awareness that the productivity gains seen since the Green Revolution with the elite lines have been at the expense of the environment and human health [[1]](https://www.zotero.org/google-docs/?y0kbSh). Moreover, these methods show their limits in reaching yield values that have hardly changed since the 1990s [[2]](https://www.zotero.org/google-docs/?alr7eA). With these constraints growing in number, varietal mixtures make it possible to optimize several parameters at the same time, in terms of drought and disease resistance, productivity and yield stability [[3]](https://www.zotero.org/google-docs/?F5GAeB). Varietal mixtures are assumed to have higher yields than their monoculture components, consistent with the theories of facilitation and niche complementarity. So far, varietal mixtures have given inconsistent results, and where both positive and negative mixture effects have been reported [[4]](https://www.zotero.org/google-docs/?SunSo2). In order to be able to design the best mixture possible, we need to understand the way plants interact with each other. Drought stress is one of the first limitations to global agricultural production due to the challenging water-limiting environment and changing climate [[5]](https://www.zotero.org/google-docs/?63hs6h). The study of the behavior of varietal mixtures must therefore be carried out under limiting water conditions in order to deal with the problems that arise in agriculture. Wheat is the second most cultivated cereal around the world [[6]](https://www.zotero.org/google-docs/?pUSh5U). Durum wheat represents 5% of the total wheat crop [[7]](https://www.zotero.org/google-docs/?1UjlYv). The largest producers are the EU with 8.5 million tonnes according to the Food and Agriculture Organisation. During the Green Revolution, the introduction of nanism genes such as *RHT* allowed to significantly increase the yield of wheat. The *RHT* gene is involved in the sensitivity of the plant to both biotic and abiotic stimuli [[8–10]](https://www.zotero.org/google-docs/?61Yk7t). Therefore, the presence or absence of the active version of this gene greatly impacts the reactivity of the plant to its environment.

Our experiments aim to answer this one question : *Are there intra-varietal interactions depending on hydric conditions within durum wheat (triticum durum) ?*

**Material and methods :**

**Genetic material**

In order to determine whether it is different for a plant to grow next to an identical genotype or a different genotype, and to recall varietal mixing conditions in the field, a single species had to be considered in order to compare its different varieties.

To optimize the number of associations and the number of repetitions, the work was done on 15 genotypes from the EPO (Evolutionary Pre-breeding cOllection) bank, developed at INRA Montpellier, France. This collection counts about 180 genotypes, with a genetic distance roughly equal between every variety [[11]](https://www.zotero.org/google-docs/?RVcxD6). The 15 genotypes were selected with a consideration for the form of the *RHT* gene, also called nanism gene. Half of our varieties own the + version and the other half to own the – version.

**Experimental design**

To test if the genotype of the neighbor has an impact on the plant, experimental design was made in mirror, with two plants in one pot. This way both plants were treated exactly the same way throughout the experiment, the first one being the neighbor of the second one and vice-versa. Our pots needed to be small enough for the soil to dry fast enough, and for the plants to have a chance to interact. The selected pots are a size of 11cm by 11 by 12. We created a pattern to plant every grain the exact same way for every pot.

*Figure 1: Experimental design scheme.*

Every genotype is seen 6 times, one time in monoculture and the 4 other times with a different neighbor. This was done for two hydric conditions, half of the pots being well watered (105% of humidity in the soil, in blue in the drawing) and the other half low watered (70%, in beige in the drawing). This design was repeated three times on three identical tables , so in the end we had 270 pots and 540 plants.(See sup figure 1) The association of the plants in couples and the position of the couples within the blocks were both chosen randomly.

**Germination Protocol**

100 seeds of each of the genotypes were weighted and were placed on petri dishes with Whatman paper humidified enough for the seed to sprout. The seeds were put at a temperature of 20°C for four days [[12]](https://www.zotero.org/google-docs/?KmVjAu). Then 36 seeds of each variety were selected in regards to their weight and their sprouting. In the end, the 36 seeds chosen were the ones whose weight was closest to the average or those that had germinated the best when some of the closest to the average had rotted or simply not germinated. The seeds were placed in the soil and the weight of the dry seed was noted for every position.

**Measures**

The first few days, the size of the coleoptile is measured [[13]](https://www.zotero.org/google-docs/?aBHMaC), then when the leaf is tied, its length and width are measured. The same is done for the second leaf, then the date where the second and third leaves tied in their turn was noted [[14,15]](https://www.zotero.org/google-docs/?mzu0Ax). The last day of the experiments was the last measure of the length and the width of the 2nd leaf, and then all of the plants were cut and put into an oven at 60°C, for them to dry but not to burn. Six days later they dried enough to be weighed.

**Cleaning of the data and statistical analysis**

The dry biomass is the chosen variable because it is not specific to the architecture of the different varieties that varied a lot. We had to take into account the fact that biomass is the only variable that did not depend on the specific architecture of each variety. For example, comparing the second leaf’s length on a given day had not a very good value because all the plants were not on the same physiological stage. Also, it does not depend on the tillering of the plant.

All the cleaning of the data and statistical analysis has been done on R [[16]](https://www.zotero.org/google-docs/?pIL9zF) software. The packages used during this analysis are the following : tidyverse [[17]](https://www.zotero.org/google-docs/?YY6bmF), car [[18]](https://www.zotero.org/google-docs/?I6gHzL), ggplot2 [[19]](https://www.zotero.org/google-docs/?u4Etfw), cowplot [[20]](https://www.zotero.org/google-docs/?BWSq7k), deeplr [[21]](https://www.zotero.org/google-docs/?H7l0tp).

Data is reorganized to fit the physical reality of our experimental design, the line and column are numerotated to form a grid so that they can be considered as numeric values. This way, we can create spatial gradients to study the environmental effects (luminosity, temperature, shadows,...) Then, we generate some new variables giving us information about the plants’ genes (*RHT*) and their neighbors.

We remove some values in order to clear our dataset : When some plants did not grow at all (5.9% of our plants) or had a growth delay, thus a final biomass lower than 20 grams (1.2% of our plants, we would remove the whole couple from our dataset. We also removed the couples for which the values were mistaken (1.2% of the values). We were down to 496 plants studied after the cleansing of our data.

For the statistical analysis of the cleaned dataset, we decided to use normal linear models:

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To complete the requirement to proceed the anova test, we use the shapiro test to attest normality of the residues and Bartlett test to check the homoscedasticity of our results.

On that model we make an anova test to determine the p value of each variable. To proceed the test, we have tested first the spatials effects, represented by the “Block, Line and Column” variables. After what, we tested the “fixed” effects : “Monoculture\_Couple, and Hydric Condition”. Finally we put in the models all other variables that can be considered as random variables : “Plant genotype, *RHT* marker, Neighbors Genotype, Neighbors *RHT* marker, Seed weight”. The alpha risk we decided is the common one : 5%.

**Results :**

1. Study of our samples’ biomass

For that part the linear model used is the following one :

Dry\_Biomass[i] = Plant\_genotype [i] + Error

Our sample’s biomass varies between 24mg and 123mg and the mean biomass is 74.45mg. The frequency of biomass’s value follows a normal pattern (Sup Figure 2), this trend is confirmed by a significant shapiro test on the biomass value residuals (p=0.5722). Also, the Barlett test value is p=0.01585 which attests the homoscedasticity of our model’s variables.

1. Fixed factors linked to the experimental design
   1. Effect of the plant’s position

For that part the linear model used are the following one and all combinations of them :

Dry\_Biomass[i] = Block [i] + Error

Dry\_Biomass[i] = Line [i] + Error

Dry\_Biomass[i] = Column [i] + Error

There is no real block effet, the p value of the simple model is 0.089 which is not significant. (See Sup Figure 3). We observe two groups of values, from the lines 1 to 9 and 12 to 20. The lines 10 and 11 are not appearing because they represent the empty space between two blocks. There is no real Line effect on the dry biomass (p value > 0.05) (See sup figure 4). We observe three groups of values, from column 1 to 5, 9 to 14 and 18 to 22. The columns 6 to 8 and 15 to 17 are not appearing because they represent the empty space between two blocks / and table. There is no real Column effect on the dry biomass (p value > 0.05) (See sup figure 5). We cannot consider that environmental factors create a gradient of biomass.

* 1. Hydric conditions

For that part the linear model used are the following one and all combinations of them :

Dry\_Biomass[i] = Hydric\_Condition[i] + Error

The repartition of the plants under the two hydric conditions is very similar. The mean biomass of stressed plants is 73.56 *±* 18.96 and the mean biomass of plants with irrigation is 75.33 *±* 19.02 . The Student test between the mean biomass of stressed and non stressed plants is not significant (p value = 0.30). When an anova is applied to the linear model, it showed that the biomass of watered plants and stressed plants was significantly the same (p value > 0.05). By studying the effect of hydric stress on the different varieties, we observe that some of them seem more sensitive to hydric stress, for example the 130 and the 376 but these effects are not significant (p value (130) = 0.51, p value (376) = 0.09) . On the contrary, the variety 395 seems to have a higher biomass when put under stressful conditions (p value = 0.03)

*Figure 2: Dry Biomass according to the hydric conditions for each genotype.*

1. Studies of the plants parameters
   1. Impact of the seed’s weight on the plantlet’s biomass

We observed a high variability between seeds weights (see sub Figure 2). The p value of the anova on the linear model representing the seed weight corresponding to the plant genotype is highly significant (p<0.01). The biomass of the young wheat sprout at 21 day is strongly correlated with its initial seed weight (p value < 0,01). (See Sup Figure 6)

According to the Sup Figure 6, we observe that wheat’s seed weight seems linked to each variety’s genotype (p<0.01). The p value of the anova taking the genotype into account is also very low (p value <0,01), which means that this link between the variety and the seed weight is highly significant.

* 1. Impact of the variety on the biomass

The linear model used in that part are the following ones :

Dry\_Biomass[i] = Plant\_genotype[i] + Error

Dry\_Biomass[i]=Plant\_genotype[i] + Error

Dry\_Biomass[i]= RHT\_plant\* Plant\_genotype[i] + Error

According to Figure 2, wheat’s dry biomass seems linked to each variety’s genotype.

The p value of the anova is very low (p value <0,01), which means that this link between the variety and the final biomass at 21 day is significant.

*Figure 3: Dry Biomass according to the plant genotype and the RHT marker of the varieties. The red line represents the average dry biomass.*

We see on the figure 2 that both heavy and light plants have the *RHT* gene. The gene *RHT* has no impact on the production of biomass (p value >0,05 for every model).

1. Impact of the neighbor interactions
   1. Monoculture vs Couple

The linear model used in that part are the following ones :

Dry\_Biomass[i] = Monoculture\_Couple[i] + Error

Dry\_Biomass[i,j] = Plant\_genotype[i]\*Monoculture\_Couple[j] + Error

The neighbor variety doesn't seem to have a big impact on the production of biomass in general. The p value of the anova is high (p value < 0.05), which means that the general link between the cultural condition (monoculture or heterogeneous couple) and the production of biomass is not significant.(Figure 3) The fact that a wheat plantlet grows better next to a neighbor of the same variety or another variety depends on which couple is considered. In most cases, the difference between monoculture and couples is not significant. But some varieties like the 118, 131 and 395 seem to produce more biomass when they are in monoculture. When we have a closer look at varieties such as 118 (p value <0.01), 131 (p value =0.20) and 395 (p value=0.12). (See sup figure 7)

*Figure 4: Dry Biomass according to the Cultural conditions for each plant genotype.*

* 1. Neighbors *RHT* marker

The linear model used in that part are the following ones :

Dry\_Biomass[i] = *RHT*\_neighbor[i] + Error

Dry\_Biomass[i,j] = *RHT*\_plant[i] \* *RHT*\_neighbors[j] + Error

Dry\_Biomass[i,j] = Plant\_genotype[i]\**RHT*\_neighbors[j] + Error

The neighbors bearing or not the gene *RHT* do not seem to have any impact on the biomass production of the focal plant. In fact we observe no global change due to the presence of the neighbor having or not the gene *RHT*. The anova has a p-value >0,05 in every model, which means that in general, there is no impact of this specific gene on the neighbour’s growth. There might be a tendency concerning the response to the neighbor’s *RHT* gene of the variety 348 which seems to be heavier when next to a *rht* - individual. 5 varieties out of 15 are only facing *RHT* + or *rht* - individuals, which keeps us from analysing their reaction to the other types of neighbour. If we isolate that 5 genotypes, and proceed another time the models. It is still not significant. (p value >0,05)

* 1. Neighbors genotype

The linear model used in that part are the following ones :

Dry\_Biomass[i] = Neighbors\_genotype[i] + Error

Dry\_Biomass[i,j] = Plant\_genotype[i] \* Neighbors\_genotype[j] + Error

The impact of the neighbor’s variety on the production of biomass is globally not significant, when you take all the values together (p value >0,05) in general. Even when focusing on the phenotypes for which the previous figures showed a tendency, the difference is not statistically significant : 118 (p value >0,05), 131 (p value >0,05), 348 (p value >0,05), 395 (p value >0,05) (See sup figure 8)

**Discussion**

This paper aimed at bringing out the interaction between durum wheat varieties in different hydric conditions.

Our results showed that at the early stage for most of the durum wheat varieties, the biomass is not influenced by the neighbor’s variety. However, the reaction to the neighbour really depends on the genotype. The biomass varies according to the variety of the plant. Each variety will have a different tissue production, but in general a plant growing next to a clone or next to another variety produces a significantly similar amount of biomass. This result meets up the previous papers that had been published in the litterature. Indeed, Helene Freville’s team explains that it is not possible to determine if plants interact and what are the underlying mechanisms associated [[22]](https://www.zotero.org/google-docs/?NO9V8a). However, our results showed that the importance of the interaction with the neighbor mostly depends on the variety of the focal plant. Our plants might be too young to already interact with their biotic environment, their roots not developed or spread enough. Maybe for some varieties, the competition or the facilitation appears in more advanced physiological stages. Only the genotype 118 showed a significant effect from its neighbors after 21 days of growth. Indeed, this variety has a significantly superior biomass when cultivated in monoculture. For his thesis, G. Montazeaud found significant results that the varietal mixtures outperformed their monoculture component by 4% [[23]](https://www.zotero.org/google-docs/?IPtmQX). Yet, he admits that other results show that overyielding highly depends on the experimental conditions [[4]](https://www.zotero.org/google-docs/?JfpZ7J). It could be more depending on the interactions between the varieties and their physical environment rather than the plant-plant interactions between the varieties themselves. Some mixtures of varieties raised the general biomass and some others did not. Studies on a larger number of varieties are needed to build a usable varietal mixture.

The early vigor is an interesting point to take into account for the choice of a future crop because it raises the competitiveness of the plantlet towards weeds. A low competitiveness in a field is a trait that is sought for in monoculture. Early vigor can be estimated by the aerial biomass, the area of the leaves, and the growth rate in our experiments. The problem is that the varieties have different architectures and different development strategies. Some varieties will differentiate their leaves early, before they grow, while others will first increase in length and width before differentiating. Therefore, comparing the growth dynamic among the varieties seems irrelevant, and the final values are the most representative of the difference between the plants. For example, biomass is allegedly not affected by the architecture differences and thus can be considered as a proper indicator of the early vigor.

Water stress is a factor that reduces the final biomass of the plant [[24]](https://www.zotero.org/google-docs/?bHC79n). Yet our results do not show the effect of water deficit. This might be due to the fact that the stress was not intense enough regarding the low water needs of the plant at that stage. Indeed, the intensity of the water stress, leading to a pressure of -4 bars at the level of the roots, was intended to be light to limit the mortality of our samples. Moreover, we chose to irrigate all the seedlings in an identical way the first days to increase the germination of the latest seeds, which could further limit the effect of water stress. The variety 395 has a significantly unexpected behaviour concerning water : the biomass of our samples is higher when they are low watered. This could be explained by the fact that this variety is stimulated by some stressful conditions. But most likely, this shows that our conditions were not stressful enough for the plants to be impacted by the lack of water.

Another unexpected result is that *RHT*, which is a nanism gene, has no impact on the biomass. The main hypothesis is that the dwarfism gene does not affect biomass allocation during early life stages, but does affect biomass allocation at later stages. Furthermore, the *RHT* bringed abiotic insensitivity hypothesis is not seen in our results. One hypothesis is that the watering difference was not marked enough to make the plant change its behaviour. Also, according to our experimental design, some plants had only a single type of neighbor, either *RHT* + or *rht* -, so our results only take into account 5 varieties. Unfortunately even if we isolate these 5 varieties we can’t show an effect of the *RHT* marker on the Dry Biomass.

**Acknowledgments**

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