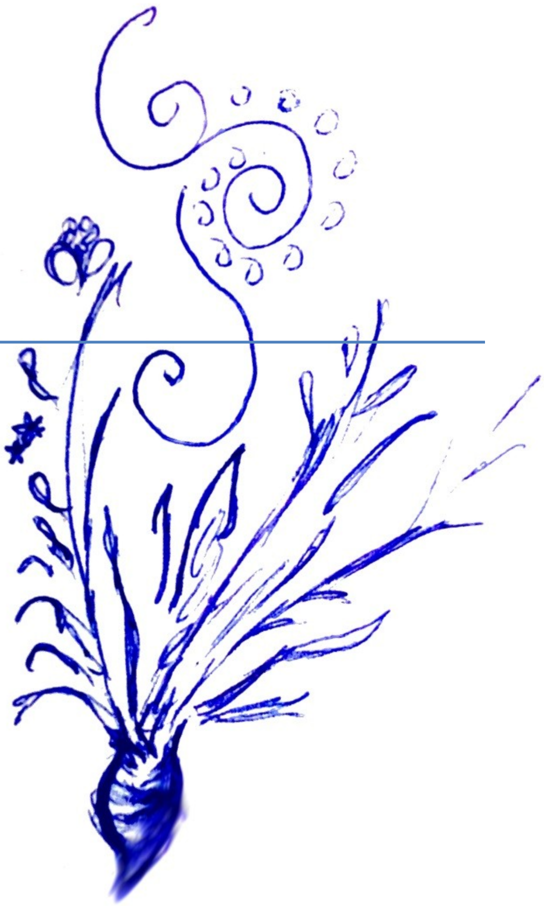
Plant Research (NZ) Ltd Internship Report

Research of new screening tools

for daikon radish breeding



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Context and introduction:

Plant Research is a small plant breeding company based in Lincoln university campus, New Zealand. Mainly peas, oats, triticale and wheat are bred for New Zealand and overseas markets. Besides, the firm has an important activity of nursery and trial managing for North hemisphere companies. Due to the size of his business, Adrian Russell is always willing to find new ways of innovation in his breeding programs and evaluation methods to improve their efficiency.

Daikon type radish (*Raphanus sativus*) is used internationally as a cover crop (Gruver et al.), reported benefits include weed competition, glucosinolates, nutrient accumulation and soil structure improvements (Extension, America research based learning network). Bio drilling has been studied for several species and radish is reported to be more successful in penetrating deeper into hard ground than rapeseed and rye (G. Chen and R. R. Weil). Some varieties of daikon radishes have already been released on the market and claim to have an improved bio-drilling capability (Tillage Radish Resource Guide). Selection of radish for use as a cover crop option in arable cropping rotations is being conducted by Plant Research (NZ) Ltd. Useful phenotypes require long roots that are able to penetrate soil pans to improve soil structure and capture nutrients for storage over the winter months, preventing leaching.

This internship aimed to identify potential selection tools for the identification of an ideal phenotype with simple and cheap methods. Plus, a replicated field trial was achieved to evaluate and compare several commercial radish varieties on their ability for nutrient accumulation.

I- Experimental material and methods:

Except nutrient accumulation, our main goal in this project was to compare existing varieties concerning "biodrilling", identificate related morphological features and define efficient tool selection if possible. Variables of interest for root growth were not known at first because no other experiment on the subject was found in bibliography. Consequently we wanted to prospect on a maximum number of different measures and evaluate their link to our traits which are mainly root volume and length.

Root growth has been studied under a succession of glasshouse experiments, followed by data collecting from two field trials for several reasons : To get root growth data from standard conditions in glasshouse, to compare values and variability between glasshouse and field, to obtain a range of growth stages from several weeks to more than 2 months.

1) Glasshouse experiments :

Plants were planted with seeds in individual pots, tubes or bags with a daily watering and fertilization every two weeks. Harvest was processed by opening the container, carefully removing ground and washing roots. Leaves have been collected as well.

Exp 1: Substrates experiment, in tubes

First reasonable scale experiment investigated radish growth in three different substrates: potting mix, perlite and a mix of sand and perlite (50/50). The goal here was to find a new substrate more homogenous and less sticky than potting mix, which would be more appropriate for harvesting of taproot and side roots. 27 individual plants were sown in plastic tubes (30cm height, 10cm diameter) : 3 varieties (Lunch, Defender and KZ7) x 3 Substrates x 3 replications.

After two unsuccessful planting for germination problem due to mineral substrates, seed couldn't had a significant growth delay in perlite and sand/perlite compared to potting mix. We suspect the problem came from an early drying of perlite which is essentially a hydroponic substrate. However, potting mix individuals have been kept and replications were harvested at different dates for comparing growth stages. It was empirically determined that the maximum duration for growth in glasshouse was between 40 and 48 days, after what radish start flowering because of the limitating size of containers.

Exp 2: variety comparison in bags

Experiments 2 intended to measure directly and indirectly root growth strength and speed. Bags were chosen with quite a small diameter to measure circumference extension under root growth pressure and hypothetically discriminate varieties. Bags were transparent in order to observe apparition of side roots. They were covered with black wrap to prevent light from disturbing side roots growth.

Experiment was designed as a randomized set of bags :

- 4 varieties : Lunch, KZ7, Black Radish and Defender
- 2 harvesting dates
- 3 reps

24 plants in total were planted in long plastic bags (43cm high, 32.5cm of circumference).

Measures taken : Above ground length, top diameter, length above 4mm wide, tap root and leaf area (picture analysis), thickness of root wall and core, sampling of those parts for comparing fresh and dry weight, take three parts of the plant for dry matter analysis (full leaves, tap root above ground, tap root underground).

However, circumference measurements couldn't be achieved as there was no extension of bags. Weight had too much residual variability to give an interesting measure of total root growth.

Exp 3: Experiment on breeding lines with commercial references

Here experiment was designed for testing extreme phenotypes from breeding populations and see how they fit hypothesis from previous experiments. Line 47 had a long inside ground root with dense side root network, whereas 154 was way out of the ground with few thin side roots.

- 4 replications by commercials (lunch, defender, black) as checks.
- 8 replications for 47 and 154.

Radishes were grown in tubes like in Exp1.

We wanted to validate:

- If diameter tool was working for extremely different lines in a same way
- If root length above ground was linked with side roots density. That's why we took these extreme lines.
- If root length above was decreasing penetration inside.

2) Field Experiments:

Both fields were submitted to a soil analysis before planting to control nutrients levels. Important levels of potassium were found with a quite high pH but no nutrient was limitating for the growth.

Commercials evaluation trial:

Experiment consisted in a fully replicated trial composed of three commercials with different features and markets orientation: Lunch, Defender and Black Radish. Annex 1 shows the design, with a randomization of replications and 2 buffer rows on the extremities of the plot. Crop was grown over spring under a full disease and weeds management program consistent with standard commercial practices for radish in New Zealand. The main goal was to analyze nutrient accumulation but data have been collected for root growth study as well. As 40 plants were grown on each rows, an assumed representative sample of 8 plants of each rows have been randomly harvested constituting a batch, separating roots and aerial part. Then roots and leaves of each batch have been mixed up and grinded with a crusher. Paper bag have been filled with samples of those and have been weighted before being dried out in ovens at 60 degrees for 2 days. Finally, those dried samples were weighted and sent for nutrient accumulation analysis to a specialized laboratory.

Breeding populations trial:

Breeding populations have been grown in a neighbour plot at the same time in same conditions. Radishes were grown in 2m long single rows. Populations were developed from hybridizations between the commercial varieties of first trial. 32 roots of reasonable size have been harvested, washed and measured for our growth study.

For all experiments, data was analyzed using R© for first selection multi plots and linear model analysis, Excel© for most graphs, and Matlab© programs were coded for picture analysis.

II- Study of a New screening tool for cover crop radish breeding

In this paragraph, upstream work to select top root diameter as a screening tool of interest will be developed. For further study on the validity of this tool with more data, the expended article is to be read.

The first question in the beginning of the study was to determine a good trait of interest for bio-drilling efficiency. What came up at first while looking at some others commercial varieties technical presentation (tillage radish) was the interest of a long root penetrating deeply the ground to grow through the plough pan in order to restore ground permeability. A first experiment was designed at PRL before the internship to measure root growth strength. Root ability to penetrate a hard clay disc introduced at a determined depth in a tube filled up with potting mix was evaluated for three varieties of radishes. However, result showed no significant difference between those varieties and all of them could penetrate the disc except for one at maximum depth. Now then, it seemed a tough problem to keep on looking for measuring growth strength. Granted this, it was more experimentally feasible to assume that growth strength is linked to speed of growth. Consequently, we chose root length, weight and volume as traits of interest.

The second question was how to investigate on a predicting tool of these traits. A prospective phase of analysis was initiated with a set of small scale experiments which intended to get data on a lot of parameters. Variables observed were: above ground length, leaves weight, diameter at top, larger point and middle bottom, tap root area, leaves weight, volume and number of side roots, above ground and underground dry matter. At this stage, there was only a few numbers of observations for each variable. Consequently an ACP would have leaded nowhere but a multiplot (each variable, even weight, volume and length are represented versus others in matrix of plots) showed some interesting trends that and let us eliminate some variables. Glasshouse experiment 1 and 2 gave more repetitions and a study based on excel graph let us chose the best correlated variables to our traits of interest.

- Lower diameters were no more explicative than top diameter.
- Side root number showed no interesting link and was too hard to measure.
- Weight was easier to get than root area and more fitted to volume.
- Length above 4mm was chosen rather than full root length : easier and more pertinent for bio-drilling purpose.
- Leaves weight showed no significant correlation with volume/weight underground, there was important residual variability and a varietal aspect.

- Side root weight and volumes couldn't be kept because of the barks sticked into it which couldn't be removed. This extra weight was biasing measures.

III- Study of the OutRatio :

The **OutRatio** has been defined as :

Root Length above ground / Root Length above 4mm wide

This ratio gives an indication on % of root which is useful for digging purpose.

1) A genetico-environmental model for OutRatio

An important question to which it was important to answer was : Is OutRatio a genetic or environmental? In other words, is root growing out of the ground because it is physically imposed to do so or is it its biological program? This feature is crucial for our subject : Assuming the root externalization is a physically controlled phenomenon due to root growth in volume and ground resistance, then a root with no good anchorage will not be efficient for bio-drilling. Attempts for comparing root growth in different substrates were not successful, so we couldn't study OutRatio sensibility to substrate resistance. Consequently our study will be statistically based on data collected from all varieties in both glasshouse and field.

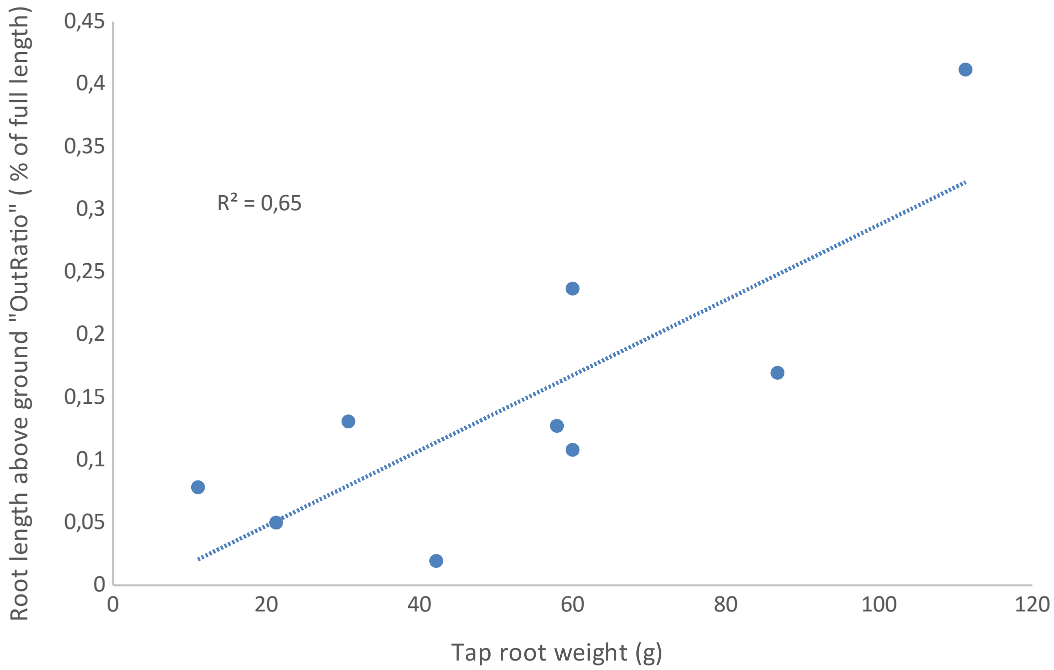

Figure 1 Data has been collected from glasshouse experiment. Variety is lunch, the reference. The adjustement of 0.653 shows a link between the root weight and the OutRatio.

Figure 1 is showing how root growth impacts clearly OutRatio. We find lower coefficients of determination in the field ($R^2 = 0.26$ for OutRatio vs Weight, 0.2 for OutRatio vs diameter), probably due to the heterogeneity of ground hardness, but the trend stays the same. As we have seen earlier, root weight is a good estimator of root volume. Consequently, at this stage we assume that encumbrance and ground hardness result, at least for a part, in root expulsion of the ground. To be more accurate on the encumbrance factor, we have to use another indicator of root encumbrance, which is tap root weight underground. Indeed, following our hypothesis, outside volume doesn't impact OutRatio so a part of our factor is unable to explain our phenomenon. Genetic can still have an impact though. We will try to identify the significance of genetic to explain OutRatio.

We define an linear model for analysis of OutRatio in **Experiment 2** :

$$\textbf{(1)} \qquad Y_{ik} = w + u_i + e_{ik}$$

Where Y_{ik} is the OutRatio of the k^{th} root of the variety i. w is the weight inside ground, a quantitative factor. This factor is called "Poids_sous_sol" in the following results. u_i is the qualitative effect of the i^{th} variety, called "variete". e_{ik} is the residual of the i^{th} variety and k^{th} observation. e_{ik} are considered independent and following a centered normal law of same variance σ. This assumptions are checked on figure 2.

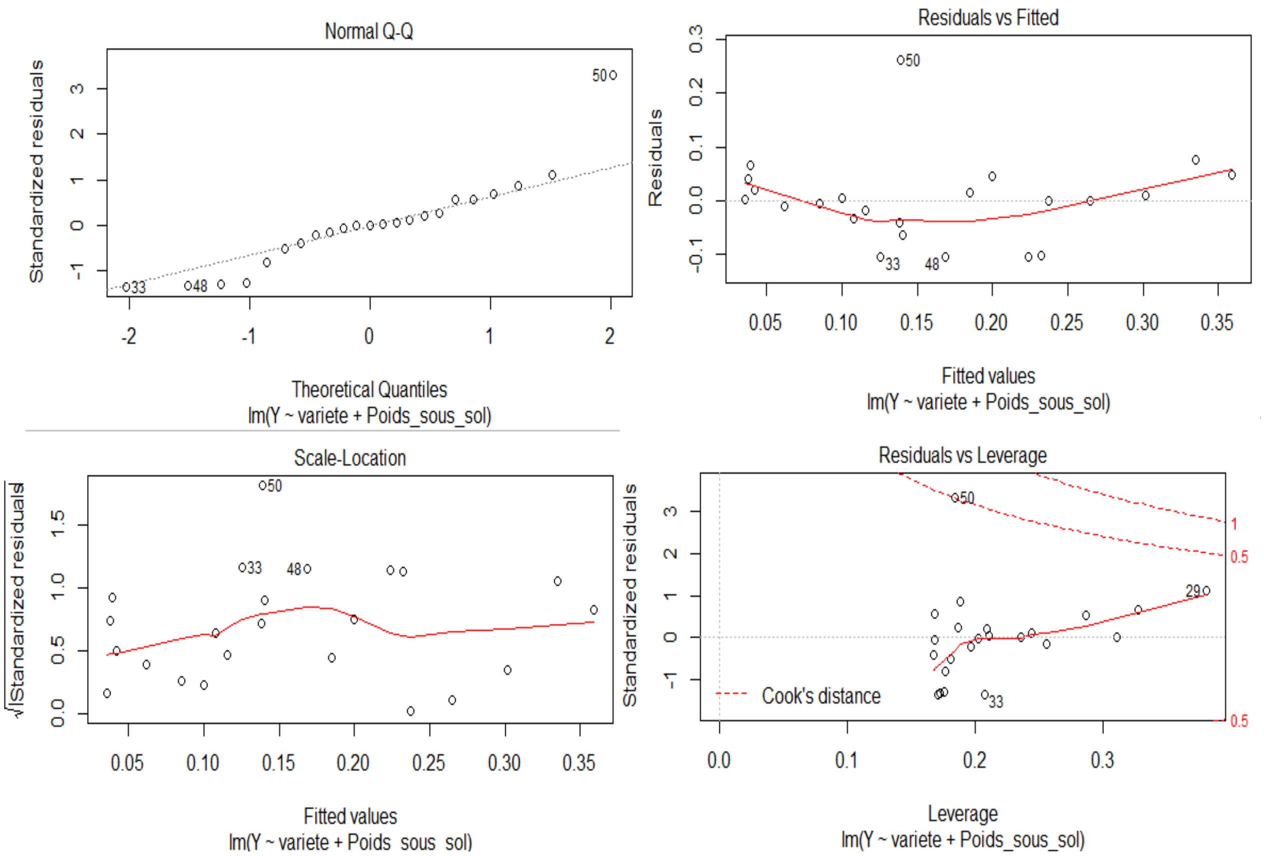

Figure 2 These graph were generated from hypothesis check of model (1). QQ Plot is describing the validity of normal distribution of residuals. Residual vs fitted helps detect a deviation to homoscedasticity hypothesis and Residuals vs leverage shows abnormal dots.

It is shown here point 50 goes against hypothesis of normality (QQplot) and is underlined as abnormal considering the graph residual vs leverage. For the sake of model validity the point has been removed from the analysis.

Then, figure 3 shows estimates results and variance analysis.

```
Call:
lm(formula = Y ~ variete + Poids_sous_sol, data = tableauAb)

Residuals:
      Min        1Q    Median        3Q       Max 
-0.102353 -0.034942 -0.003128  0.039411  0.085038 

Coefficients:
                 Estimate Std. Error t value Pr(>|t|)    
(Intercept)    -0.0402980  0.0343560  -1.173    0.257    
varietedef     -0.0354053  0.0337613  -1.049    0.309    
varietek27      0.0454616  0.0337257   1.348    0.195    
varietelunch    0.0368512  0.0353836   1.041    0.312    
Poids_sous_sol  0.0048076  0.0005934   8.102 3.07e-07 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.05566 on 17 degrees of freedom
Multiple R-squared:  0.8116, Adjusted R-squared:  0.7673 
F-statistic: 18.31 on 4 and 17 DF,  p-value: 5.434e-06
Analysis of Variance Table

Response: Y
               Df   Sum Sq  Mean Sq F value    Pr(>F)    
variete         3 0.023548 0.007849   2.534   0.09133 .  
Poids_sous_sol  1 0.203347 0.203347  65.647 3.069e-07 ***
Residuals      17 0.052659 0.003098                      
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Figure 3 Coefficients present the estimated values of model effects, the intercept correspond to variety black radish. Respectively under we find values of σ estimation (Residual standard error), R^2, and at the bottom is the Analysis of Variance which shows the components of variability with significance.

Underground weight ("Poids_sous_sol") effect is very significant and has a positive effect on the OutRatio (Y). Besides, the varieties effects are not significant and explain a small variability. However, the whole variety factor as a slightly significant effect which indicates at least one of the genetic effects has probably a different average than others. Sum of square ratio for underground weight indicates it explains more than 70% of the variability observed, whereas it is 8.4% for variety. Regarding this data, underground weight is strongly impacting the OutRatio. Its effect is superior here to genetic , even with these 4 different varieties.

Model (1) applied to **Experiment 3** has shown less significance but growing method was different and lead to a superior residual error. However, may be a more repeated experiment would show a significant genetic effect.

2) Impact of Side Root density

We now want to study the effect of another parameter on the OutRatio. In a perspective of a breeding program, we want to understand if a good side root network can prevent root from being pushed out of the ground. So measures of the side root density have been taken. A matlab program has been created to calculate side root area showing up on the side of the potting mix. Visual result of Side Root area can be observed with figure 4.

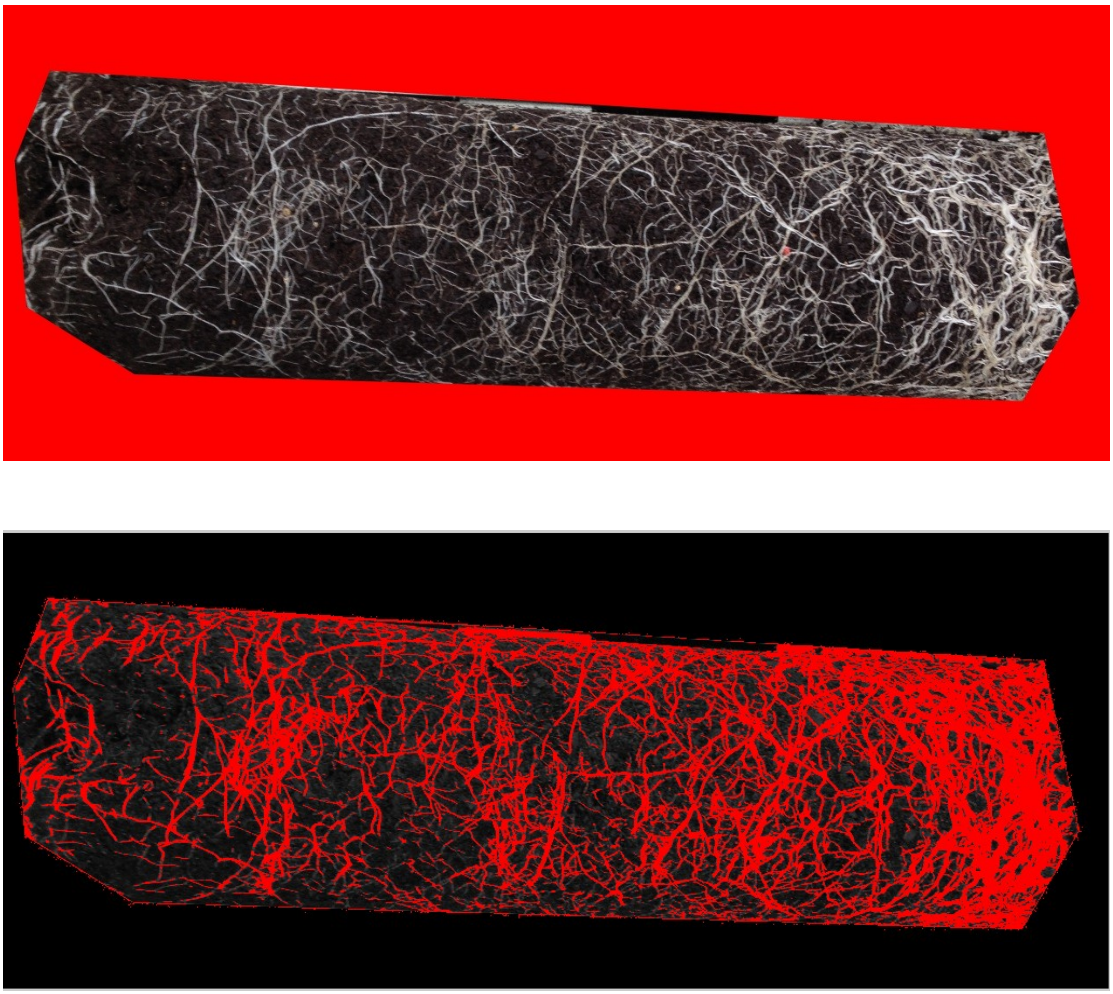

Figure 4 At the top we can see picture of the intact substrate straight after the tube opening. The picture after scan is shown at the bottom, side roots are materialized in red. The density is calculated by the ratio of red on the area of potting mix (the pitch black around is not part of it).

For the purpose of finding a distinction between varieties for side roots density, a statistical comparison of varieties by pairs has been achieved. The null hypothesis of this test is : "Average side root densities of the two varieties compared are equals"

We have to note this kind of test overestimate the significance of each test because it assumes the two varieties compared are the only ones that have been evaluated in the experiment. However, numbers of varieties remain reasonable here (4 in Exp1, 5 in Exp2) and we can consider the whole result significant at a risk of less than 10% (cumulation of risks) for the second line (α=1%). However, first line is much more hazardous.

	B<K	B<D	B<L	K<D	K<L	L<D
Test α=5%	NO	YES	NO	YES	NO	YES
Test α=1%	NO	NO	NO	NO	NO	YES

Experiment 1

	B<L	B<D	B<47	154<B	L<D	L<47	154<L	D<47	154<D	154<47
Test α=5%	YES	YES	YES	NO	YES	NO	YES	NO	YES	YES
Test α=1%	NO	YES	YES	NO	YES	NO	YES	NO	YES	YES

Experiment 2

"Yes" means "shows a significant difference", and α is the risk of second type chosen for the test (it means : the risk to reject null hypothesis even though it is true). Experiment 2 has been more discriminant than experiment one. For both experiments, differences detected show visually a difference of OutRatio.

There is another way to look at it to compare encumbrance and side roots effects. It is to inspect the link between these parameters setting a multiple regression model like in (1), replacing genetic effect by Side Root density.

$$\textbf{(2)}\quad Y_k = w + d + e_k$$

Like in (1), Y_k is the OutRatio, w is the underground weight and e_k is the residual error. Here, d is the side root density.

Results: In exp 1, SR has a slightly significant effect but explains only 3% of variability. In exp2, where model has not a good adjustement (R^2=0.1674), SRdensity is significant at 5% and explain 15,5% of the variability observed, which is 12 times more than underground weight.

To extend the study to the field, graph in **Annex 2** shows correlation between data obtained from glasshouse experiments and field trial 1 for Lunch, Black radish and Defender. X axis is represented in a logarithmic scale to makes reading easier. Glasshouse radishes have general higher OutRatio (curve are higher on Y axis) but the trends are fitting between varieties. Field data shows much more variability than glasshouse because of ground variability.

The conclusion is side root density would be worth repeating the experiment with more replications and a standard method. Side root network is a complex trait to study experimentally, and side root density is only an indicator with important residual variability.

However, we can be confident that side root network is most likely to be the main influent parameter on root resistance to ground extraction while growing. Then, we can classify our varieties in term of anchorage like follows:

154 < B < L = K < 47 = D

Varieties have been ordered following their Side Root Density average and regarding the significance of their differences.

47 has been chosen for the glasshouse trial because it was well inside ground in the field. It is a line coming from a defender cross. As many of the roots observed in the field seem to demonstrate it, root profile is in some aspect a heritable trait, especially in the case of side root network. All cross from defender show this characteristic to have plenty of strong horizontal roots. At the contrary, crosses from lunch and other varieties without important side root network happen to have this main smooth taproot and have a superior OutRatio.

As parental origin is not a fully determinist scheme for root shape, a sample of 32 roots have been digged out from the ground among early generation breeding material. Individuals observed in nursery had root shape diversity. They have been classified regarding this aspect.

- **Post:** The root consists in a big vertical tap root and thin side roots.
- **Fork post:** There is no real taproot but several big roots have developed at the same time. They can be straight, slanted, coiled in helix, but are too big to be seen as side roots. Is probably a reaction to hard ground or stones, with a genetic predisposition for black radish.
- **Vertical scavenging:** The root has a conical sheaf of reasonable size secondary roots from the tap root bottom. Could be a reaction to the hard ground, or genetic.
- **Horizontal scavenging :** The root has reasonable size sides roots horizontally in the ground from tap root, can go as far as 40cm with a diameter > 4mm. Could be a reaction to the very hard ground, but seems more common in defender and his related crosses. So it is probably mainly a genetic aspect.

Pictures of each category are shown in Annex 3.

Defender trend to behave as a D, Lunch as an A, Black goes often B. There could be seen as genetic root traits, amplified in a hard ground context. These behaviors impact as well on root pushing up the ground. Type A is more likely to be pulled up as growing big, whereas type D stays more easily in the ground. For our purpose we should be looking at a D or C root, which could dig deep and prospect independently of the hardness of ground.

Now if we group similar shapes A-B (few side roots) and C-D (good side roots scavenging) to compare anchorage properties:

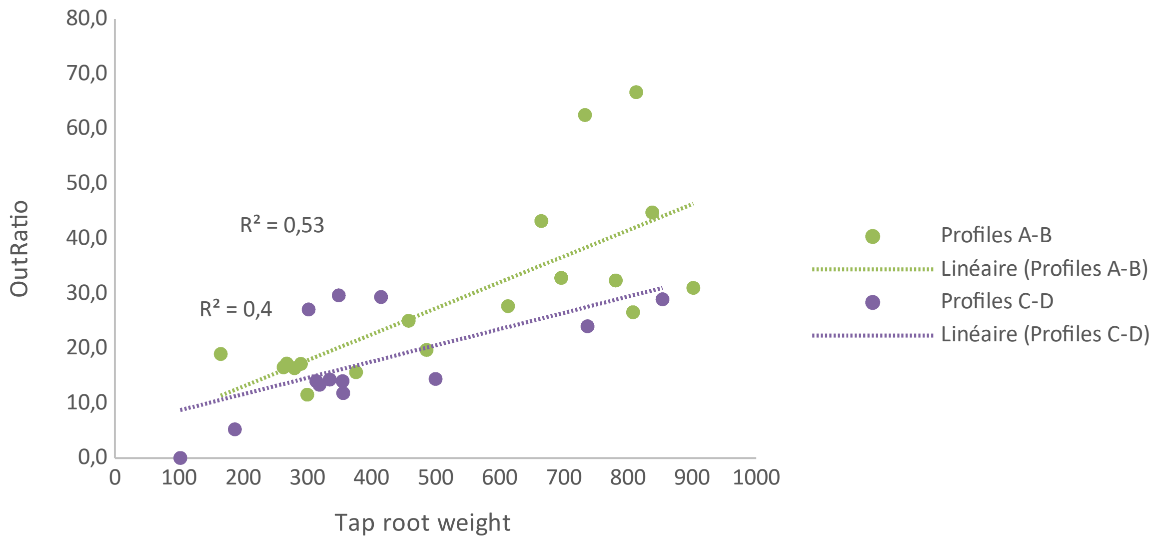

Figure 5 Shows the same correlation as in the field experiment. Plus, we can see C-D profiles tend to have a higher percentage <u>in</u> the ground while growing.

This graphs confirms side root profile favor anchoring even with a heterogeneous and more mixed genetic than for the commercials.

See graph at Annex 3 for a spatial representation of In-Out root lengths for several varieties.

As a conclusion, the root's shape plays an important role in anchorage of the plant, phenomenon which takes place in hard grounds and increasingly during the growth of the root in volume. This way, for an efficient and regular bio-drilling in a radish variety, we don't only expect the root to be long but to have a good side roots network. By the way, we not only increase anchorage but nutrients prospection with a better spatial covering. A reduced root length above ground is a good indicator of side root network , as long as we seek for a thick top diameter as well, which indicates us the size of the root.

IV- Comparative trial of three radish varieties for nutrients accumulation:

Result of accumulation was obtained for 14 nutrients. The analyze purpose was to detect significant distinctions between varieties and quantify nutrient accumulation at an agronomic scale.

1) First remarks on accumulation values

High level of potassium was found in plants. It is explained by the high level already present in the ground as it appears on soil analysis. We found as well high levels of iron without significant differences between varieties for that. Concentration in leaves is highly variable as well, regardless of varieties. This must be the result of an environmental cause, probably from the history of these fields or the process of the sample like some rust in the crusher used after harvest. Mustard catch crop showed nitrogen level of 2.03 without fertilization and 2.39 with (Ninane et al.), which is coherent with our radishes (same brassica family).

2) Analyse

As three groups are to compare, it is better to use an anova type method than a comparison of pairs, which would overestimate the significance of the test.

Hypothesis required for tests have been checked out and normal repartition was validated for every treatment. Variances estimations showed reasonably similar values for more than half of nutrients and plants can be considered independents because of the randomized sampling during harvest in every row (We consider most plants harvested where not affected by the others harvested in the row and obviously even less by the others out of the row).

For each test, our null hypothesis is: Ho = “All group averages for concentration in X are equals”

We are going to test this hypothesis for our 14 nutrients. For this purpose we use a Fisher statistic which compare variability between group to variability inside group with the following expression :

F = CMinter / CMintra

It is basically the ratio of squares average between groups and squares average inside groups. Finally we compare this statistic value to a reference from table for risk α=5% and α=1%. Results are summed up on the following table. We can see the averages concentrations for each variety/nutrient. Colors indicate significance of the test. Bright colors mean Ho was rejected with risk α=1%, pale ones correspond to α=5% and white means Ho was kept. Red means a significantly higher concentration, blue is lower and when two cases have the same color on a same test, it means we can’t discriminate these two.

	Variety	Nitrogen %	Phosphorus %	Potassium %	S %	Ca %	Mg %	Na %	Fe mg/Kg	Mang mg/Kg	Zn mg/Kg	Cu mg/Kg	Boron mg/Kg	Crude Protein %	Soluble Sugars %
Roots	Lunch	1,5	0,29	6,05	0,36	0,65	0,19	0,23	181	12,3	29,5	2,25	22,3	9,73	3,68
	Defender	1,43	0,35	6,38	0,38	0,55	0,14	0,26	274	15	37	3	21,5	9,35	1,43
	Black Radish	2,95	0,61	7,43	0,73	0,59	0,23	0,16	244	17	49,3	4,25	31,5	19,6	3,73
Leaves	Lunch	1,98	0,32	3,35	0,57	1,66	0,23	0,66	333	21,5	25,8	4	28,5	13	2,68
	Defender	1,95	0,38	4,23	0,61	1,37	0,23	0,18	206	23,3	35	4,75	30,5	12,9	1,9
	Black Radish	3,28	0,44	6,95	0,73	3,22	0,55	0,31	408	33,5	39,3	6,75	56,5	21,5	1,68

We can see leaves and roots results correlate pretty well and Black radish shows a higher concentration in nutrient for a lot of nutrients. This is probably partly due to the late development stage of this radish. At harvest time, Black radish was still in vegetative growth with much less visual biomass than Lunch and Defender that where already flowered.

3) Extrapolation to the field

We use measurements of fresh weights after harvest, dry matter ratios and known spacing distances of planting (40cm spacing on the row) to determine nutrient accumulation at the scale of the field and obtain the following results.

Accumulation per hectare (kg/ha)

	Nitrogen	Phosphorus	Potassium	S	Ca	Mg	Na	Fe	Mang	Zn	Cu	Boron	Crude Protein	Soluble Sugars
Lunch	238	39	435	67	194	27	77	3936	255	319	47	344	1561	337
Defender	254	50	596	79	172	30	26	2853	300	472	61	396	1673	25
Black radish	105	18	247	25	56	12	8	1056	80	155	18	141	696	10

Defender shows up to be the more efficient in accumulation/ha except for Calcium, Sodium and sugars where Lunch beats it. Let's not forget Na adds salty taste and sugars makes it sweet, it is probably not a coincidence as Lunch is a human consumption variety. Levels of iron are incredibly high, which makes sense with our previous warning on this nutrient. Black radish is really lower than the two others varieties. Black radish was very late in development, especially for leaves biomass which was a quarter of the others in average. Consequently, even if it has a higher concentration in nutrient, it doesn't make enough biomass to compensate so there's nothing surprising in these results. We can suspect defender's good side root network participates to its efficiency in nutrient scavenging. This feature of good nutrient accumulation, especially for negatively charged ones (N, P, S…), is of great interest to prevent leaching during autumn and winter season.

Conclusion

Breed radishes on their root features without digging them out can't be an easy task. However, we showed top root diameter is an interesting tool for screening high volume radishes. By drawing upon reduction of above ground length and crosses from varieties with good side root network, breeding is heading for radishes with better bio-drilling features. May be more advanced research could be able to answer to the real mechanism of root growth strength. Besides, we saw Defender had interesting properties regarding nutrients accumulation, another quality whose must be taken in consideration.

As a sum up of the internship itself, working on the project and especially writing a scientific paper learnt me about what rigor and deepening takes research in biology. The internship interest was not only scientific but above all let me discover the world of a small breeding company, where working on every kind of task helped me take a step back on what is Plant Breeding in others dimension than research. Plus, it was instructional to see how innovation can be achieved at this scale by taking a different approach than in a multinational group. Finally, to land in a new linguistic and cultural environment at work was personally enriching.

Acknowledgments

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Cheers.

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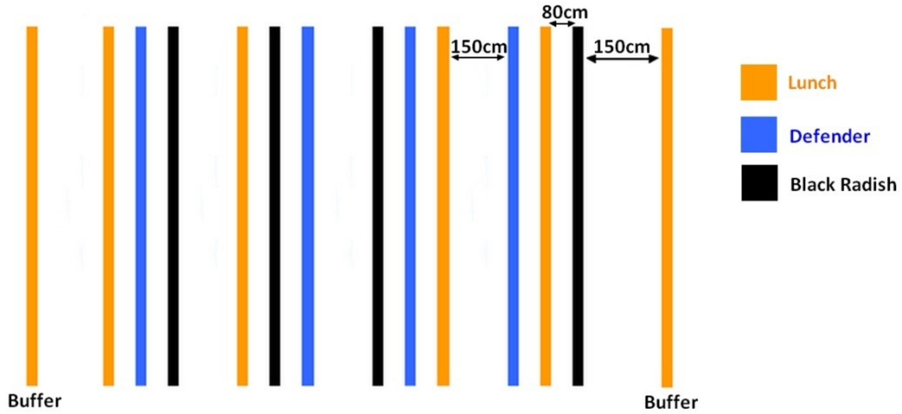
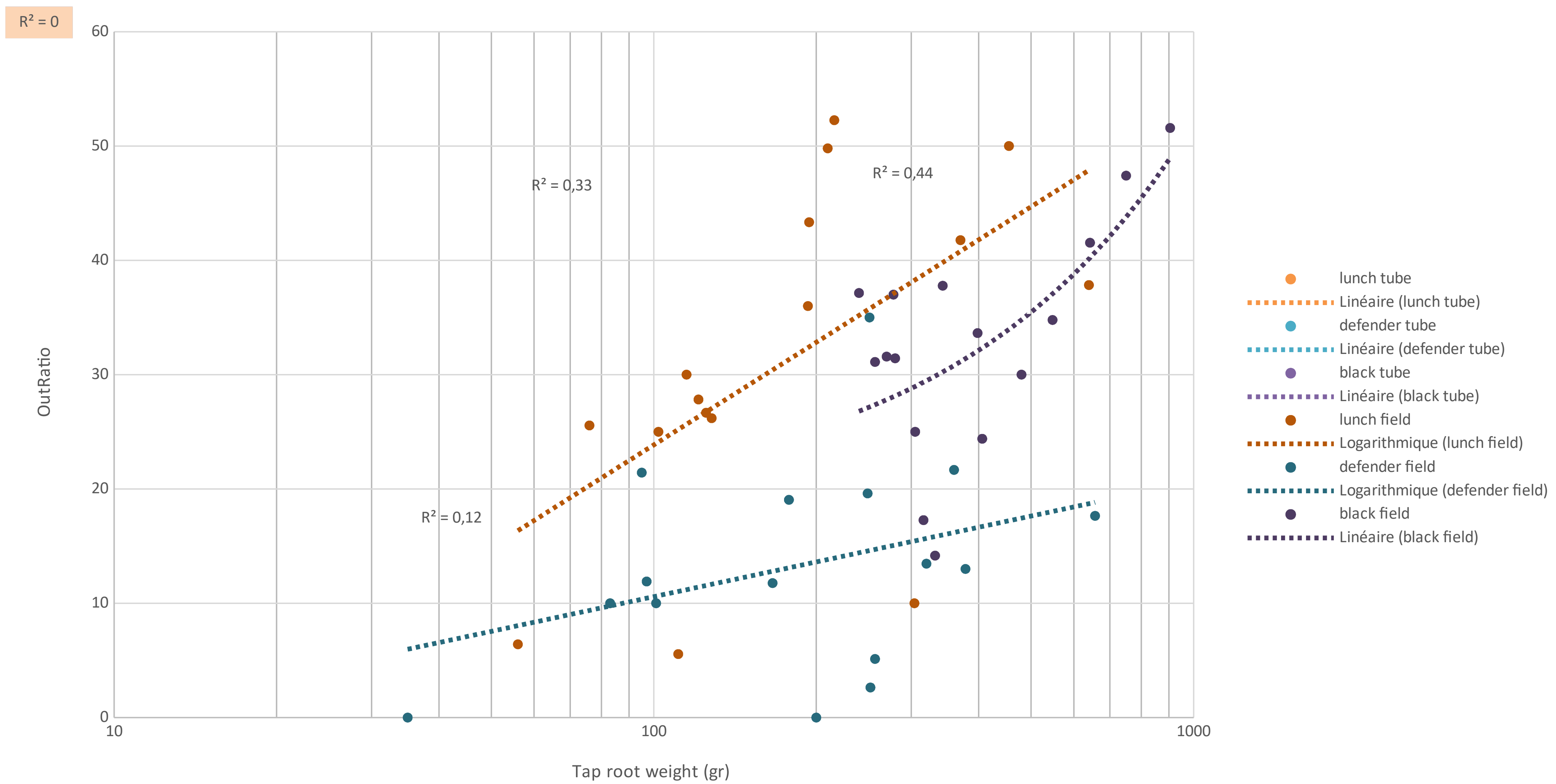
Annex



Annex 1

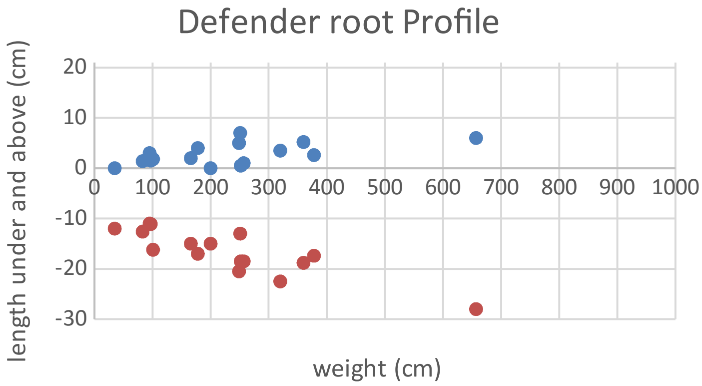


R² = 0
60
50
40
30
20
10
0
OutRatio
R² = 0,33
R² = 0,44
R² = 0,12
10
100
1000
Tap root weight (gr)
lunch tube
Linéaire (lunch tube)
defender tube
Linéaire (defender tube)
black tube
Linéaire (black tube)
lunch field
Logarithmique (lunch field)
defender field
Logarithmique (defender field)
black field
Linéaire (black field)

Annex 2

Annex 3

Annex 4


Defender root Profile
length under and above (cm)
20
10
0
-10
-20
-30
0
100
200
300
400
500
600
700
800
900
1000
weight (cm)