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Intra-specific differences of non-structural carbon investment and DBH variability of *Pinus nigra* in common garden experiment

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Abdullah-Al Mamun

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SUPPLEMENTARY INFORMATION

LIST OF ABBREVIATIONS

ANOVA = Analysis of Variance

Compnt = Component

DBH = Diameter at Breast Height

Df = Degree of freedom

Df den = Degrees of freedom of denominator (also called error degrees of freedom)

e.g. = exempli gratia means “for example.”

i.e. = id est, means “in other word”

LS Mean = Least square mean

NSC = Non Structural Carbohydrates

P = Probability of significance

$\mathbf{P_{annual}}$ = Mean annual precipitation

PCoA = Principal Coordinate Analysis

REML = Restricted Maximum Likelihood

SS = Soluble Sugar

$\mathbf{T_{max}}$ = Mean maximum temperature in summer (June, July and August)

$\mathbf{T_{min}}$ = Mean minimum temperature in winter (December, January and February)

$\mathbf{T_{range}}$ = T_{max} - T_{min}

Var = Variance

Growth Rate = $(DBH_{2017}-DBH_{2016})/ DBH_{2016}$

Abstract

Increased non-structural carbohydrates (NSC) storage and decrease growth is considered one of the important responses of trees under different biotic and abiotic stresses, which may drive either genetically or environmentally. Higher genetic diversity and phenotypic plasticity of Mediterranean black pine (*Pinus nigra*) may possibly have different responses in NSC storage and diameter at breast height (DBH). The purpose of this study is to investigate the nonstructural carbohydrates storage (in small branches), as well as DBH variability among 18 provenances of 3 sub-species of *Pinus nigra* in test site with uniform environmental condition. Colorimetric method was used to measure starch and soluble sugar concentration (percentage of dry weight basis) from powdered branch sapwood samples collected in mid-July, 2017. Climatic data of the origin of provenance was collected from KNMI Climate Explorer. In general, percentage of soluble sugar was less than starch and they are highly correlated independent of provenances and sub-species category. Only percentage of starch is significantly different among 18 provenances, however, percentage of total NSC, soluble sugar and starch were significantly different among 3 sub-species level. DBH also vary significantly in both provenance and sub-species level, whereas growth rate (2016-17) was not varying significantly. Climatic variables (mainly T_{max}, T_{min}, T_{range} and P_{annual}) on the geographic origin of provenances have influence in NSC accumulation in branch sapwood. Provenances originated with low T_{max} and T_{range} have significantly lower NSC investment in comparison with provenances originated at similar or high T_{max} and T_{range}, however, P_{annual} have significantly negative relation only with soluble sugar investment, whereas, DBH nearly significant positive correlation only with T_{max}, furthermore, provenances of lower latitudinal origin have significantly higher percentage of soluble sugar and DBH than provenances higher latitudinal origin. Overall study reveals that differences in NSC accumulation and DBH of provenances in test plantation site partly regulated by heredity and growth phenology on the basis of their climatic and geographic origin, as well as, their phenotypic response to environmental stress in test site.

Key word: *Pinus nigra*, DBH, non-structural carbohydrates, Mediterranean, provenances, soluble sugar, starch, climate change.

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Chapter 1: Introduction

Carbon is the building block of all life. Forests is considered as an important sinks for atmospheric CO_2 and storing carbon in plant biomass, detritus, and forest soils. Trees maintain a complex mode of allocation of photosynthates. Decrease in primary production is expected due to a lack of quick adjustment to changes in their surrounding and they are more severely affected by environmental constraint. Trees growing under stress conditions are damaged due to less favorable carbon balance causing drop of productivity, growth and reproduction (Larcher, 1995).

Nonstructural carbohydrates (NSC) in tree tissues reflect the relative balance between source (net canopy assimilation) and sinks (the use of assimilates for growth and respiration) (Hoch et al. 2003). NSC are directly or indirectly involved in plants functional processes, mainly of primary and secondary metabolism (Hartmann and Trumbore, 2016). Under normal conditions, tree partitioning the current photosynthate allocation for various functional priorities such as structure (lignin and cellulose), protection (phenolics), metabolism (nitrogen compound, organic acid and lipids) and storage (starch and soluble carbohydrates); not only that, partitioning of dry matter also depending on tree age. The relative weight of crown, stem and root system vary in accordance with age. In young trees a very high portion of photosynthate is used in leaf production, in contrast, most of the dry weight of old trees are in the main stem and comparatively less in the crown and root system (Kozlowski et al. 1991).

As Mediterranean biomes are considered to be one of the most exposed to potentially harmful effects of climate change (Giorgi and Lionello, 2008). The mechanism of plants carbon allocation and store for growth and survival under upcoming climate change scenario are getting more and more attention (Dietze et. al. 2014). It is expected that, Mediterranean pine forest may be submitted to serious biotic and abiotic risk driven from climate warming. There is an increasing concern of possible climate change which is impacting in present and also will impact in future forests because more frequent and intense droughts are predicted, particularly in the Mediterranean basin (Pachauri et al. 2014). Since woody tissues constitute the main terrestrial biotic pools for long-term sequestration of atmospheric CO_2, identifying which factors control tree growth as well as carbon reserve variability is crucial for improving projections of the effects of climate change on forest productivity (Delpierre et al. 2016).

Maintaining a positive carbon balance during drought is one of the major physiological challenges for trees (Mitchell et al. 2013). Although a large storage of carbon is maintained in tree tissue, tree growths can be limited by failure to use non-structural carbon to growth (sink limitation) or availability of carbon within the plant (source limitation) (Hoch, 2015); because of that, carbohydrate concentration in tree tissue frequently used as a measure of environmental and physiological constraints on plant yield (Tjoelker et al. 1999).

There is also evidence that tree actively stored NSC in living tissue in expense of growth to cope with unpredictable future climatic conditions (Genet et al. 2010) and due to increase need of osmoregulation (Sala et al. 2012). It is already known that, cell growth and differentiation are more sensitive to drought stress than photosynthesis (Lempereur et al. 2015). Carbon starvation history of past and present may have influence on tree’s conservative allocation strategy that augmented reserve formation in expense of growth (Wiley and Helliker, 2012). Therefore, an increase in carbon storage and decrease in growth could be a plastic or evolutionary response of tree to carbon-limiting conditions.

Black pine (*Pinus nigra*) and other conifers are characterized by pronounced seasonal changes of NSC in different functional unit level. NSC concentrations have been measured in different tree organs, including stems, branches, foliage and roots with a general conclusion of low variation was recorded within the storage tissues mainly in sapwood, coarse roots and ray parenchyma and a high intra-annual variation of NSC content observed nearer to the active growth sites (e.g. apical, root meristems and vascular cambium) (Simard et al. 2013).

Branches are one of the most important functional units of the tree supporting apical buds, leaves and other reproductive organs and act as channel for transporting photosynthetic assimilates to other plant organs as well as transport water and nutrient to the leaves. Though carbohydrates dynamics in branches of conifers are less pronounced but generally they follow the same dynamics as of needles (i.e. increased starch accumulation before bud break followed by depletion of starch towards the end of growing season) (Hoch et al. 2003). However, carbohydrates accumulation also vary depending on numerous internal and external factor such as plant position, timing (day or season), water potential (Woodruff and Meinzer 2011), fertilization, drought, temperature, pollutions and CO_2 concentration, and reproduction (Oleksyn et al. 2000). Branches are usually considered as autonomous in carbon supply (Cregg et al. 1993) and in comparison with roots, stems and foliage (in conifers); NSC pools in branches are relatively small and short term in nature (Hoch et al. 2003). According to Hartmann and Trumbore (2016) despite its major role in plant functions and the stand-level carbon cycling our understanding on NSC dynamics, its controls, effect on growth and response to environmental stresses are still limited.

Increasing carbon storage along with decreasing growth can either be controlled genetically or environmentally, and in most cases these two drivers are difficult to separate (Granda and Camarero, 2017). To test the carbon storage difference between populations, common garden experiments could be used as measure to compare NSC concentration relative to growth rate (Wiley and Helliker, 2012) and their nature of responses reflect the adaptive potential of tree (Franks et al. 2014).

Tree species that are adapted to diverse environmental condition with high genetic diversity are expected to be prioritized in future forestry program (Thiel et al. 2012); in this prospective *Pinus nigra* is one of the most important conifers of southern Europe distributed about 3.5 million ha from Europe to Asia Minor and North Africa (Oliva et al. 2006).

Bioclimatic conditions of different sub-species are usually not similar but most of the black pine sub-species are growing in Mediterranean-type climate, except *Pinus nigra nigra* which is more typically temperate. Though, morphological and genetic markers have confirmed the common phylogenetic origin of all black pines but, due to wide natural distribution, differentiation of population/ sub-species level categories are emerged which are not fully agreed by the various authors.

Most commonly six sub-species are described: *Pinus nigra mauretanica* presence only in Morocco and Algeria; *Pinus nigra salzmannii* (Dunal) Franco presence from the Pyrenees to Andalusia in Spain and with a few isolated populations in the Pyrenees and Cevennes in France; *Pinus nigra laricio* (Poiret) presence in Corsica, in Calabria and in Sicily; *Pinus nigra nigra* found from Italian Apennines north of Greece through the Julian Alps and the Balkans; *Pinus nigra dalmatica* found only in few islands of the coast of Croatia and on the southern slopes of the Dinaric Alps; and finally *Pinus nigra pallasiana* (Lamb.) Holmboe covers extensive areas in Bulgaria, eastern Greece and Turkey and is also present in Cyprus and Crimea (Isajev et al. 2004; Climent et al. 2013).

Among the six sub-species the most different and genetically original European groups are *P. nigra salzmanii* and *P. nigra laricio*, although *P. nigra nigra*, *P. nigra dalmatica* and *P. nigra pallasiana* appear quite similar. Genetic diversity as well as adaptive traits, within and among populations, are high in black pine, which results in a great variability of traits such as vigor, form and drought, frost and disease resistance (Isajev et al. 2004).

Mitchell (1972) described the identifying characteristics of the most divergent group. The most recognizable characteristics of *P. nigra salzmannii* are its broad, slightly depressed branching with fine, pale leaves, while white buds and orange shoot are common features of the foliage. Shoot become pale pink in third year and cones are smooth. Growth rate moderately rapid in young but slow when old; however, *P. nigra laricio* have the columnar crown and short, light, level branches. In the foliage, the pale yellow-brown shoot and long leaves are distinct among 2-needle pines. Growth in height is maintained well to 25-30 meters and growth in girth is rapid for about 50 year; on the other hand, *P. nigra nigra* distinguished from var. *laricio* (Corsican pine) generally by the heavily branched wide crown and darker bark but the aspect of the foliage is different usually in dense bunches separated by short bare lengths of shoot, the bunches opaque and blackish, with straight, short needles. Height growth of first 5-6 year is higher than Corsican pine but rapidly falling behind after that. However, despite its great ecological and economic potentialities, *P. nigra* is still one of the European less studied conifers.

This study examines the variability of carbohydrates (soluble sugar and starch) accumulation in branch and DBH of different provenances of *P. nigra* in common garden condition, as well as, carbon recourse investment variability in relation to climatic and geographical origin of the provenances, furthermore to investigate the response of different provenances of *P. nigra* in relation to mild environmental stress in common garden condition.

It is hypothesized that depending on the climatic conditions and wide geographic origin; adaptive genetic diversity and phenotypic plasticity of different provenances of *P. nigra* will vary in common garden uniform environmental condition, and that also generate variability of carbon resource investment as well as DBH among different provenances and sub-species of *P. nigra*

Chapter 2: Objectives

The main objective of this study is to investigate the variability among different provenances and sub-species of *P. nigra* under common garden condition.

The specific objectives are:

1. To analyze the variability among different provenances and sub-species of *Pinus nigra* in relation to the carbon storage investment (starch, soluble sugars) in branches.
2. To analyze the variability of DBH among different provenances and sub-species of *P. nigra*.
3. To examine carbon investment in relation to the climatic and geographic origin of provenances.
4. To analyze the carbon storage investment of different provenance in relation to mild environmental stress in common garden condition.

Chapter 3: Methodology

3.1. Description of the test site

The P25GRA test site, belonging to the Genfored test network (www.genfored.es) is located at 1150 m.a.s.l. at coordinates 40º 54' 41.22" N and 4º 0' 55.16" W, between San Ildefonso and the Embalse del Ponton Alto in the province of Segovia. The plot has a north-northwest orientation and a slope of 5-7% with the mean temperature of 12°C and mean annual precipitation of 541 mm of the last 30 years, shown in (Figure 1).

The trial plot includes approximately 800 individuals of 22 years old trees that were planted in 1996 after nursery. The trees are distributed in 12 blocks, within each of which provenances are distributed randomly. Each provenance is represented in each block by four trees planted to real frame.

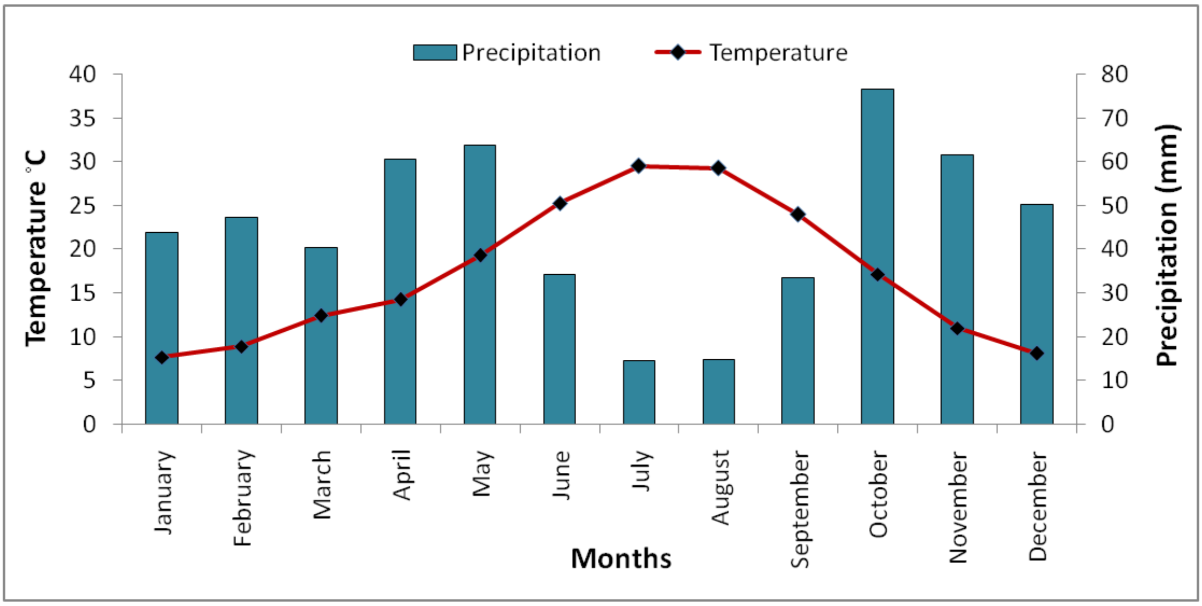
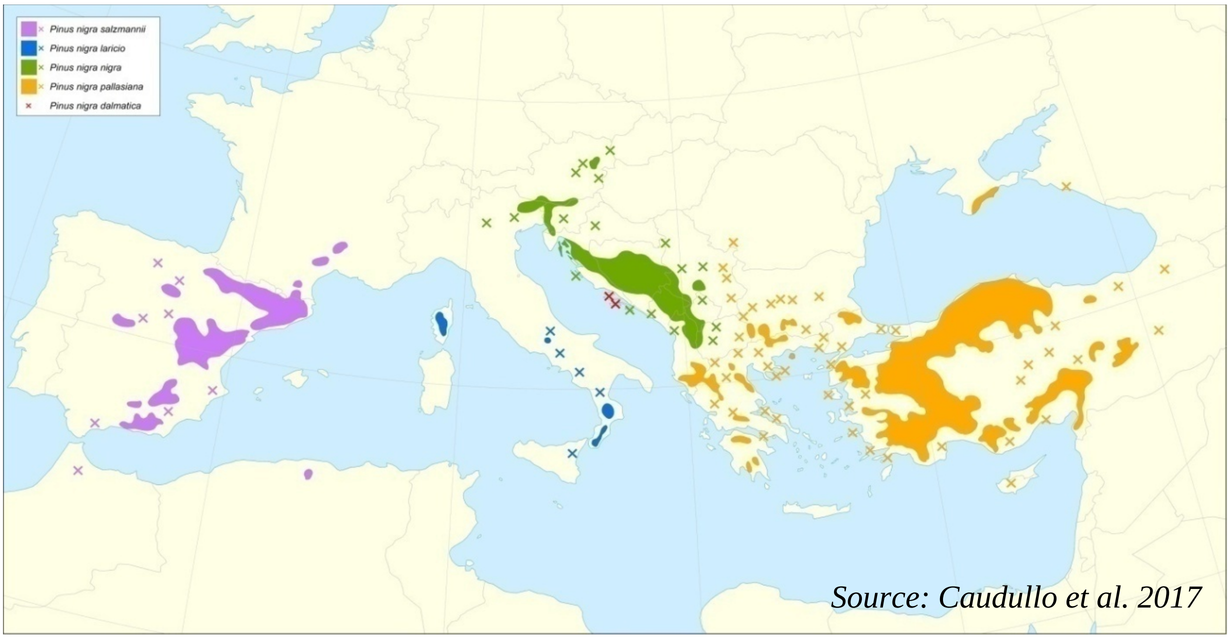


Figure 1: Climogram with monthly mean temperature and precipitation (1986-2016) in test plantation site in Segovia, Spain.

The trial comprises 19 provenances of *P. nigra* generated with seeds of natural populations of Spain, France, Italy, Austria, Greece and Romania, classified *a priori* in four sub-species (*salzmannii*, *laricio*, *nigra* and *dalmatica*); out of that, 10 provenances belong to sub-species *salzmannii* of Spanish and French origin; 5 provenances to sub-species *laricio* of

Italian and French origin; 3 provenances to sub-species *nigra* of Austrian, Greek and Italian origin and 1 provenance is a sub-species *dalmatica* of Romanian origin. Nevertheless, the geographical location of the Romanian provenance (601) is not precise, so there are doubts about its taxonomic ascription between *dalmatica*, *nigra* or even *pallasian*. In this study due to confusion in geographical location sub-species *dalmatica* (601) has excluded from analysis. Sampling was operated in the first 6 blocks out of 12 blocks from this test site. The distribution of different sub-species is shown in (Figure 2).



Source: Caudullo et al. 2017

Figure 2: Distribution map of different sub-species of *Pinus nigra*.

3.2. Climatic parameters in the geographical origin of provenances

In this study, I extracted climatic parameters data: mean, maximum and minimum temperature (T_{mean}, T_{max} T_{min}, respectively) and mean annual precipitation (P_{annual}) of the origin of tested provenances, from 1986 to 2016 period, by using GPS coordinates from KNMI Climate Explorer (https://climexp.knmi.nl/start.cgi) at 0.5° grid level resolution. Climatic parameters of the origin of provenances are shown in Table 1.

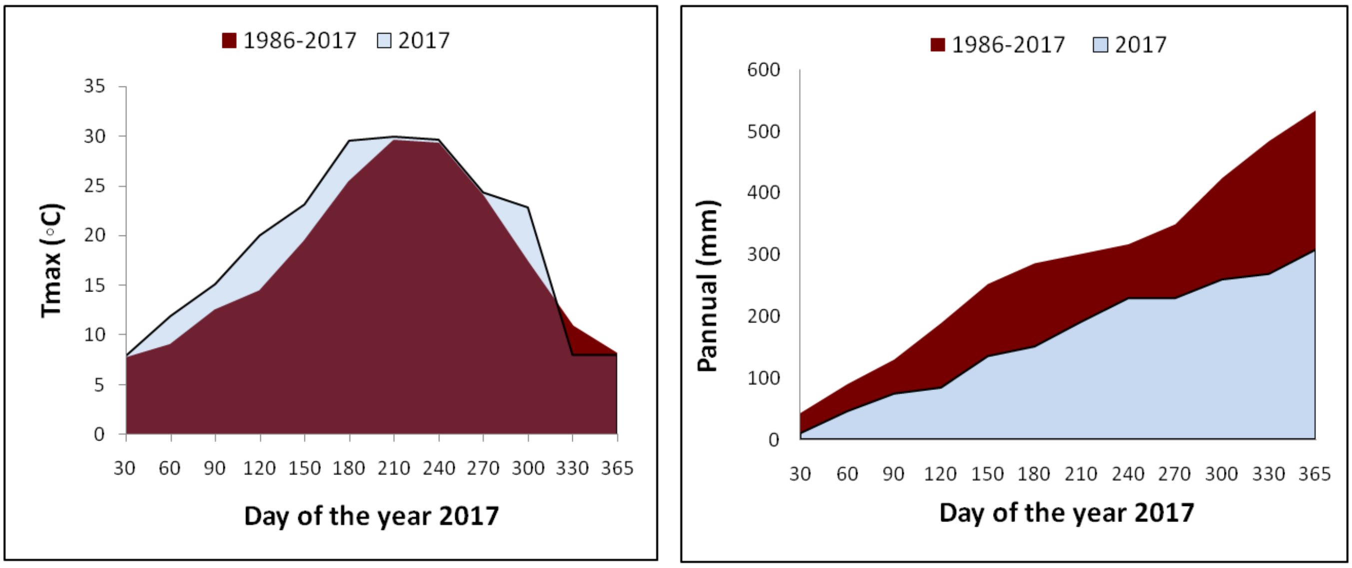
Table 1: Bioclimatic conditions of different provenances of *Pinus nigra* tested on the P25GRA site, from 1986-2016 period.

Code (provenances)	**Sub-species**	**Country (origin)**	**Elevation (m)**	**P_{annual} (mm)**	**T_{max} (°C)**	**T_{min} (°C)**	**T_{range} (°C)**
1	salzmannii	ES	800	950	22.06	-3.45	25.52
3	salzmannii	ES	770-820	818	22.71	-1.36	24.08
71	salzmannii	ES	1200	434	30.21	0.02	30.19
72	salzmannii	ES	1150-1280	532	27.54	-2.55	30.09
73	salzmannii	ES	1100	434	30.21	0.02	30.19
81	salzmannii	ES	1200-1500	426	31.04	1.34	29.70
82	salzmannii	ES	1300	413	31.06	0.13	30.93
83	salzmannii	ES	700	411	30.70	0.46	30.24
84	salzmannii	ES	1660	415	30.06	0.87	29.18
150	salzmannii	FR	200-500	847	27.55	1.72	25.84
201	*laricio*	IT	850	794	27.13	4.93	22.20
202	*laricio*	IT	950	794	27.13	4.93	22.20
302	*laricio*	FR	1000	781	22.24	2.27	19.97
303	*laricio*	FR	1350	781	22.24	2.27	19.97
304	*laricio*	FR	1100	781	22.24	2.27	19.97
402	*nigra*	AU	1713	1626	15.49	-7.12	22.62
405	*nigra*	GR	1120-1380	806	27.15	-1.82	28.98
407	*nigra*	IT	1000-1200	768	29.38	3.38	26.05
601	*dalmatica*	RU	191	626	26.55	-2.83	29.39
Test site	All	ES	1150	541	28.01	0.21	27.80

Climate data Source: Climate Explorer, May 5, 2018

3.3. Field sample collection and processing

Sampling was conducted in mid-July 2017; first DBH (diameter at breast height) was measured for each individual tree and subsequently branch samples were collected from the top of sun facing crown and manually remove the entire phloem and bark portion, immediately frozen in dry ice to stop further metabolic activity. Xylem water was extracted cryogenically, wood samples were grinded into fine powder, subsequently sieved and preserved in air tight container for further NSC analysis. It is to be noted that, the climatic condition of test site during sampling year (2017) was drier in comparison to 30 years average (1986-2016) shown in (Figure 3).



Sources: weather station of Segovia, Spain (© AEMET)

Figure 3: Comparison of mean maximum monthly temperature (1986-2016) and mean annual precipitation (1986-2016) with sampling year (2017).

3.4. Carbohydrate sampling and analysis

NSC comprises low molecular-weight soluble sugar (glucose and fructose, sucrose, and other free sugars) plus starch, and they were analyzed following a colorimetric procedure (Figure 4) described by Dubois et al. (1956) and Buysse and Merckx (1993).

Weighted powder (branch wood) samples of about 50 mg were extracted with 10 ml of 80% ethanol in a hot water bath at 60°C for 30 minutes, after centrifugation, an aliquot of the extract (300 µl) was subsequently mixed with 80% ethanol (200µl), 28% phenol (500µl) and 96% sulfuric acid (2500 µl) and absorbance read at 490 nm wave length in UV-1600PC spectrophotometer after 30 minutes to determine soluble sugar content. It is to be noted that, spectrophotometer was calibrated with blank and different concentration of standard solutions of glucose every time before taking the absorbance reading.

The content of starch was obtained from the same sample after decant all the supernatant and dried over night at 70°C and subsequently mixing 4 ml of sodium acetate (0.1 M and pH 4.5) and 1 ml of amyloglucosidase (0.5% w/v) from *Aspergillus niger* (10115-5G-F, Sigma-Aldrich) and incubate over night at 50°C to break down all starch to glucose. After that, I followed exactly the same procedure (as in the case of soluble sugar) to determine starch content. Nonstructural carbohydrates (soluble sugar and starch) in branch sapwood of different provenances of *P. nigra* were expressed in percentage of dry weight basis.

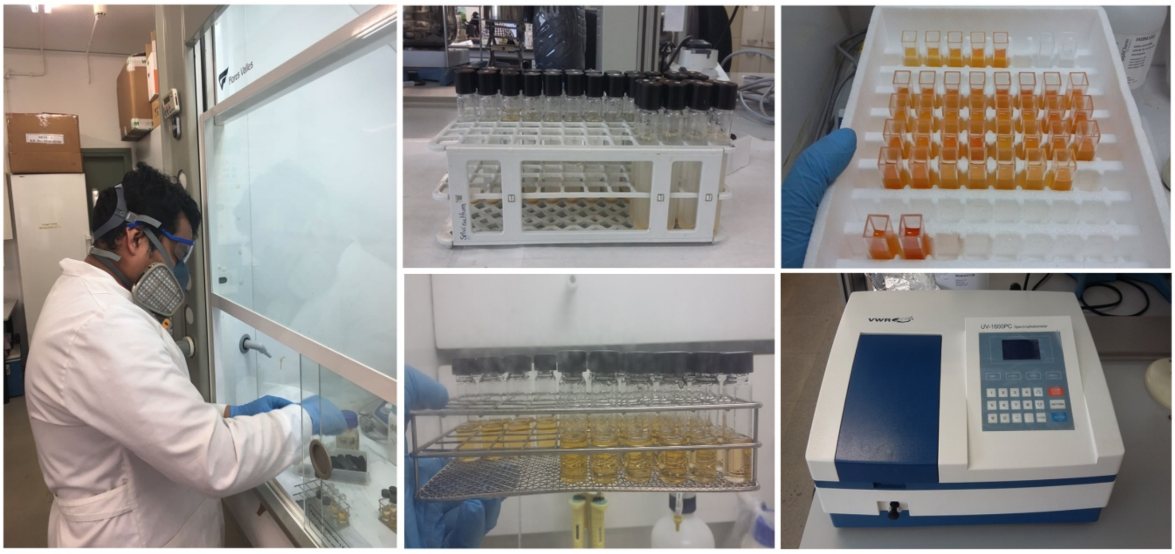


Figure 4: Determination of branch wood NSC of *Pinus nigra* by using colorimetric method.

3.5. Data Analysis

As the data of NSC (soluble sugar, starch and total) consisted of percentage values having a narrow range of variations, square root transformation was performed to achieve normality (SI Figure 1 and SI Figure 2).

The effect of provenance, block, sub-species, tree and the interaction of them on sugar, starch, total NSC, DBH (2017) and growth rate (2016-17) of 18 provenance and 3 sub-species of *P. nigra* were analyzed fitting linear mixed-effect model using statistical package JMP Pro 13 (SAS Institute, Cary, NC, USA).

To observe the effect of provenances and sub-species on NSC (soluble sugar, starch and total), DBH and growth rate a Linear Mixed-Effect Model was used as follows:

- Fixed factors: Provenance, Sub-species, Provenance [Sub-species] and Block
- Random factors: Provenance*Block, Tree [Provenance*Block]

In every case, where we found significant (or, nearly significant $P<0.1$) relations from above models, we conducted multiple comparisons using LS Mean difference of Student's T test.

To determine the influential climatic variables, we tested at provenance and sub-species level the significance of T_{mean}, T_{max}, T_{min} and T_{range} and P_{annual}, Mean summer precipitation at the origin of provenances. For further analysis, we take into consideration those climatic parameters which have a probability threshold at least 90% or more ($P \leq 0.1$).

Hierarchal clustering (ward method) was used with those climatic variables which are within the threshold probability limits and subsequently leveled by provenance code, to make a cluster of provenances with similar climatic condition in their origin.

Both simple and partial correlations of response variables (total NSC, sugar, starch and DBH) with climatic variables (within the threshold probability limits) were also conducted. In-case of partial correlation, we used sub-species as a confound variable. The main purpose of doing both the simple and partial correlation was to observe the degree of association of those variables, which may sometime mislead by simple correlation coefficient. Similar values of simple and partial correlation coefficient indicate the strength of the correlation between two variables and disparity indicates there is an effect of sub-species.

To determine if provenances are under stress in test plantation site (by increase/decrease of temperatures and precipitation), a new variable was implemented as a ratio between origin of provenances and test site's climatic parameters plot the provenances separated by test site climatic condition.

In order to analyze relationships between provenances of the samples and the measured variables, a Principal Coordinates Analysis (PCoA) was performed using a generalization of Gower's distance, since it allows the analysis of both continuous and categorical variables (Pavoine et al., 2009). Besides, patterns of similarities among provenances based on the measured variables were performed through a hierarchical cluster analysis with Ward distance. PCoA was carried out using CANOCO 5.0 (Ter Braak and Smilauer, 2012). Cluster analysis was performed using the stats package available within the R statistical environment (RDC Team, 2005).

Chapter 4: Results and Discussion

4.1. Results

4.1.1. NSC in relation with provenances and sub-species

In general, percentage of soluble sugar is less than the percentage of starch in different provenances of *P. nigra* in mid-July (Figure 5a) and among three sub-species of *P. nigra*, sub-species *nigra* and *salzmannii* have higher NSC concentration in branch sap wood than sub-species *laricio* in mid-July (Figure 5b).

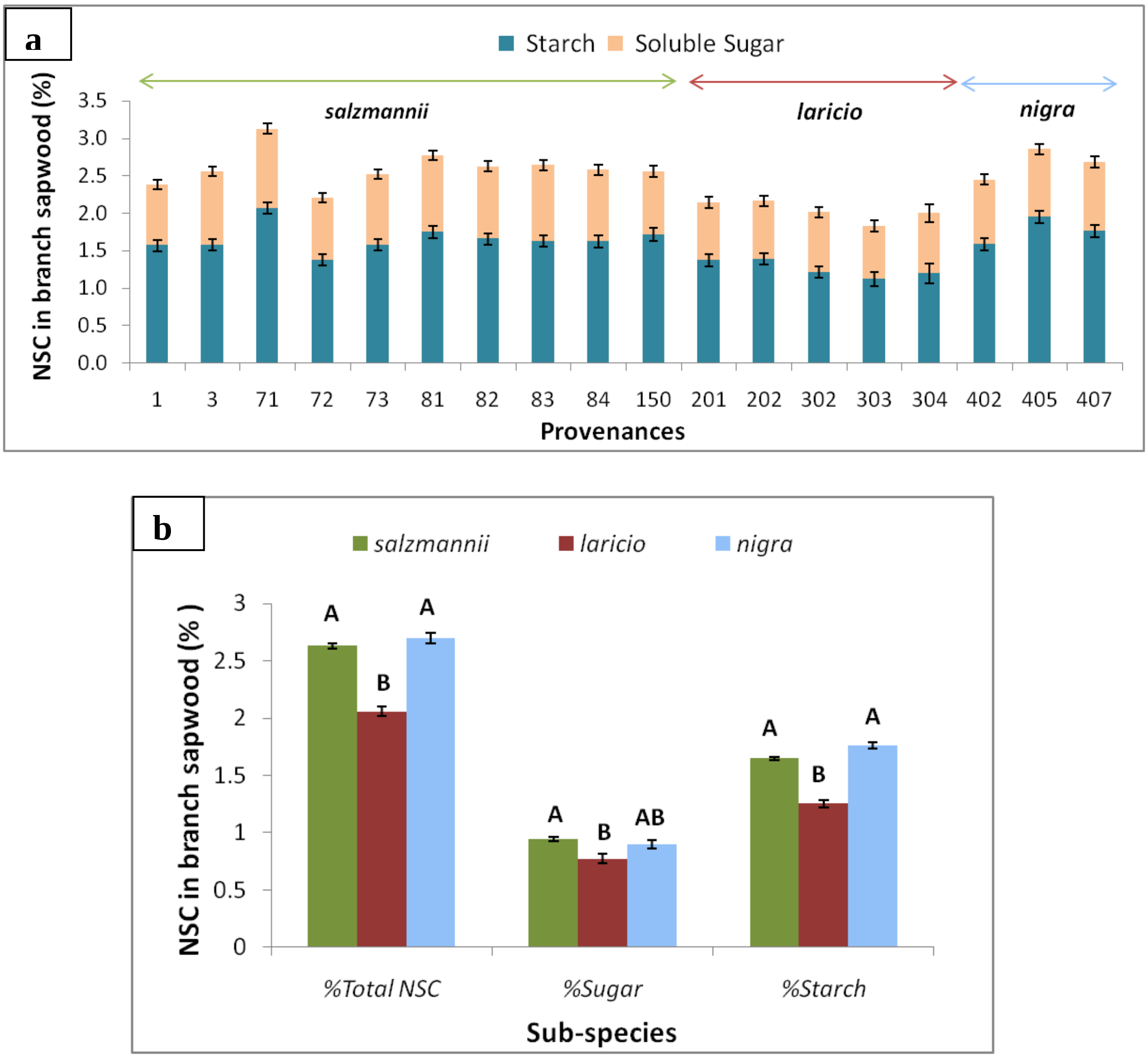


Figure 5: Total NSC, soluble sugar and starch percentage in branch sapwood in provenance (a) and sub-species (b). Different letters stand for significantly difference ($P< 0.05$) among sub-species.

Table 2: Linear mixed effect model of NSC; fixed effect: provenance, sub-species, provenance [sub-species] and block, whereas, random effect variables: provenance* block, Tree [provenance, Block].

Fixed Effect	**Total NSC**				**Sugar**				**Starch**			
	df	df den	F Ratio	Prob > F	df	df den	F Ratio	Prob > F	df	df den	F Ratio	Prob > F
Provenance	17	77.88	1.66	0.0701	17	187.5	0.94	0.5238	17	77.35	1.84	0.0373*
Sub-species	2	81.98	7.92	0.0007*	2	187.8	3.71	0.0263*	2	81.43	8.86	0.0003*
Provenance[Sub-spp]	15	77.5	0.75	0.7279	15	187.5	0.49	0.9422	15	76.97	0.85	0.6184
Block	5	77.63	4.59	0.0010*	5	187.3	3.55	0.0043*	5	77.08	4.52	0.0012*

Random Effect	**REML Var Compnt (Total NSC)**				**REML Var Compnt (Sugar)**				**REML Var Compnt (Starch)**			
	Var ratio	Var compnt	Std Error	% of total	Var ratio	Var compnt	Std Error	% of total	Var ratio	Var compnt	Std Error	% of total
Provenance*Block	0.61	0.01	0	11.06	0	0	0	0	0.47	0.01	0	10.94
Tree[Provenance,Block]	3.81	0.05	0.05	70.46	3.01	0.03	0	75.09	2.81	0.04	0.01	65.67
Residual	-	0.01	0.01	18.48	-	0.01	0	24.91	-	0.01	0	23.39
Total	-	0.07	0.07	100	-	0.04	0	100	-	0.06	0.01	100

P values < 0.05 are marked by asterisk

4.1.1.1. Total NSC

Total NSC in branch sapwood were not varied significantly (*P*> 0.05) at provenance level although it is nearly significant (*P* = 0.07), whereas, if provenances are categorized into sub-species then they are significantly (*P*<0.05) different, although provenances inside every sub-species category are not varied significantly (*P*>0.05) (Table 2). It is to be noted that, about 70% of the total variability of NSC was explained by the variability of individual tree within each block and provenances (Table 2). Mean separation test (Least square mean of student's T test) of provenances revels that, provenance 71 of Cuenca (Spain) origin have significantly higher percentage of total NSC in branch sap wood than provenance 201, 202 (Italy), 72 (Spain) and 302, 303, 304 (French) origin (Table 3 and SI Table 3), whereas, mean separation test of sub-species revels sub-species *nigra* and *salzmannii* have significantly higher concentration of total NSC in branch sapwood (percentage of dry matter) than sub-species *laricio* (Table 4). It is important to mention that, starch and soluble sugar concentration was highly correlated during the sampling period in mid-July (Figure 6).

Table 3: Least square mean difference (Student's T test) of % total NSC concentration in branch sapwood of different provenances; common or similar lettering indicates no significant differences.

Provenance code	**Ranking (Total NSC)**	**LS Mean**
71	A	3.15
405	AB	2.89
81	ABC	2.79
83	ABC	2.73
407	ABC	2.72
82	ABCD	2.69
84	ABCD	2.62
3	ABCD	2.61
150	ABCDE	2.57
73	ABCD	2.53
402	ABCDE	2.48
1	ABCDE	2.44
202	BCDE	2.24
72	BCDE	2.22
201	CDE	2.16
302	DE	2.03
304	BCDE	2.02
303	E	1.85

Table 4: Least square mean difference (Student's T test) of % total NSC, % sugar, % starch concentration in branch sapwood of different sub-species; common or similar lettering indicates no significant differences.

Sub-species	**Total % NSC**		**% Sugar**		**% Starch**	
	Ranking	LS Mean	Ranking	LS Mean	Ranking	LS Mean
nigra	A	2.70	AB	0.90	A	1.76
salzmannii	A	2.63	A	0.94	A	1.65
laricio	B	2.06	B	0.77	B	1.25

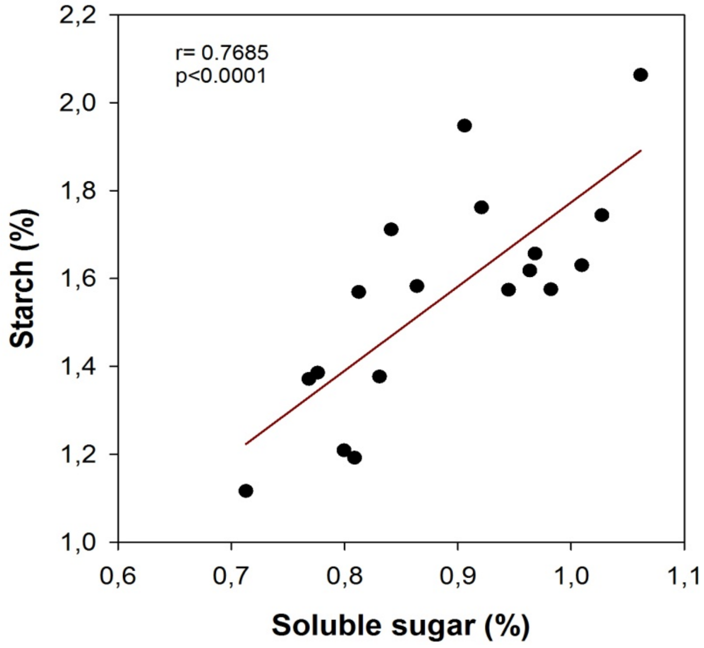


Figure 6: Correlation between starch and soluble sugar concentration in branches of different provenances of *P. nigra*

4.1.1.2. Percentage of soluble sugar

Soluble sugar (SS) concentration in branch sapwood of different provenances of *P. nigra* varied, however, it was not significant (*P*>0.05), whereas it was varied at sub-species category, although, provenances within each sub-species category were not varied (Table 2). It is to be noted that, about 75% of the total variability of sugar was explained by the variability of individual tree within each block and provenances (Table 2). Mean separation test revealed that sub-species *salzmannii* and *nigra* have significantly higher concentration of soluble sugar in branch sapwood (percentage of dry matter) than sub-species *laricio* (Table 4 and Figure 5b).

4.1.1.3. Percentage of Starch

Starch concentration in branch sapwood of different provenances, as well as, sub-species of *P. nigra* varied significantly (*P*<0.05) (Table 2). In contrast with sugar, 65% of the total variability of starch was explained by the variability of individual tree within each block and provenances (Table 2). Mean separation test revealed that, out of 18 provenances, only provenance 71 Cuenca (Spain) and 405 Milea (Greece) have significantly ($P < 0.05$) higher starch concentration in branch sapwood (percentage of dry matter) than provenance 303 of Sorba (France) origin (Table 5); whereas, sub-species *nigra* and *salzmannii* have significantly higher concentration of starch in branch sapwood (percentage of dry matter) than sub-species *laricio* (Table 4 and Figure 5b).

Table 5: Least square mean difference (Student's T test) of % starch concentration in branch sapwood of different provenances, common or similar lettering indicates no significant differences.

Provenance code	**Ranking (Starch)**	**LS Mean**
71	A	2.06
405	A	1.95
407	AB	1.76
81	AB	1.74
150	AB	1.71
82	ABC	1.66
83	ABC	1.63
84	ABC	1.61
402	ABCD	1.58
3	ABCD	1.58
73	ABCD	1.57
1	ABCD	1.57
202	BCD	1.39
72	BCD	1.38
201	BCD	1.37
302	CD	1.21
304	BCD	1.19
303	D	1.12

4.1.2. Non-independence of residuals of sugar and starch

Plantations row (Y) and column (X) as well as their interaction did not have any significant (*P* > 0.05) effect on the residuals distribution of % sugar and starch (Table 6); that means, residuals of sugar and starch are independently distributed along plantation row and column (Figure 7).

Table 6: Residuals distribution of sugar and starch along plantation row (Y) and column (X) at test plantation site

ANOVA	Residuals of % sugar				Residuals of % starch			
	df	df den	F Ratio	Prob > F	df	df den	F Ratio	Prob > F
X (Plantation column)	1	75.54	0.67	0.42	1	81.71	1.10	0.31
Y (Plantation row)	1	72.89	0.61	0.44	1	78.5	0.19	0.32
X*Y	1	85.67	0.28	0.61	1	90.56	0.85	0.36
Provenance	17	71.27	0.01	1.00	17	74.04	0.03	1.00
Block	5	71.82	0.12	0.19	5	74.9	0.21	0.16

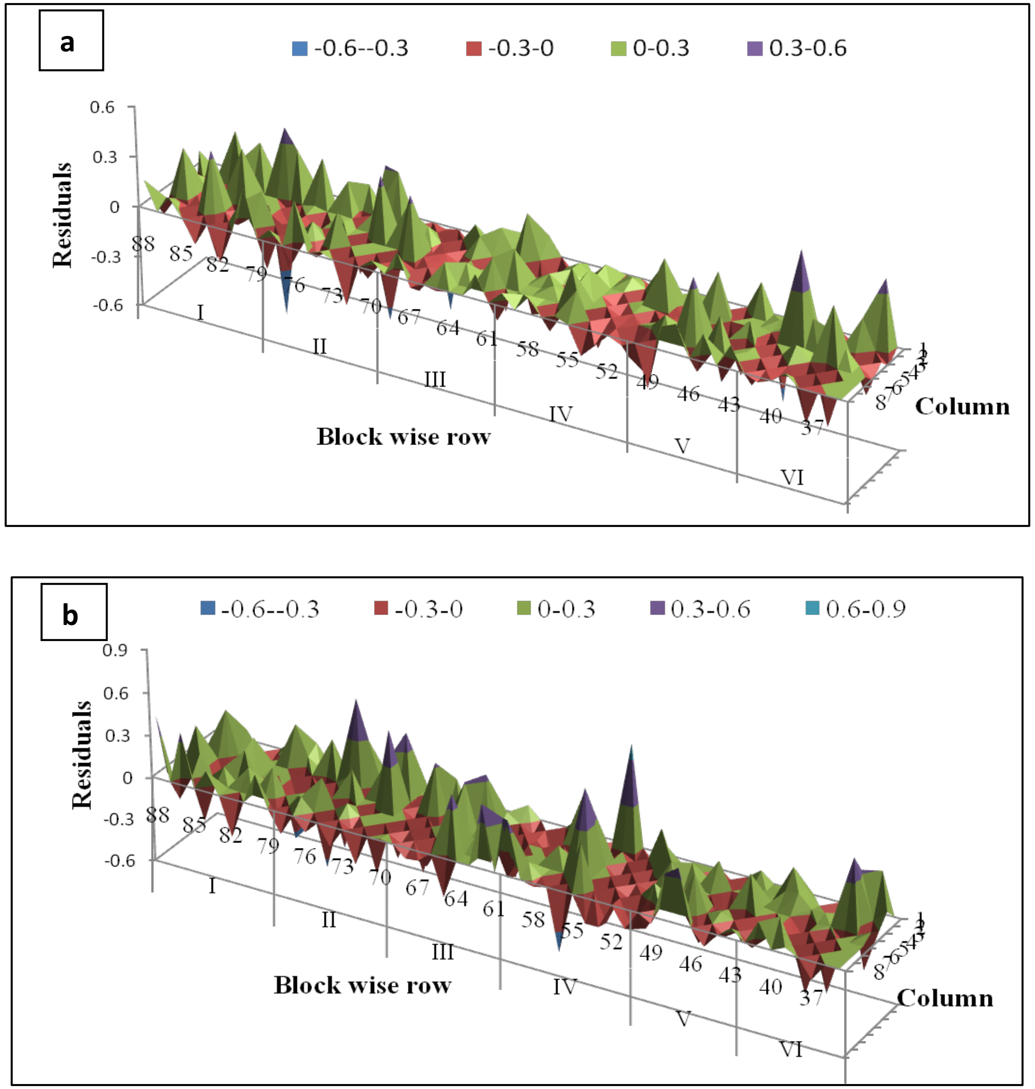
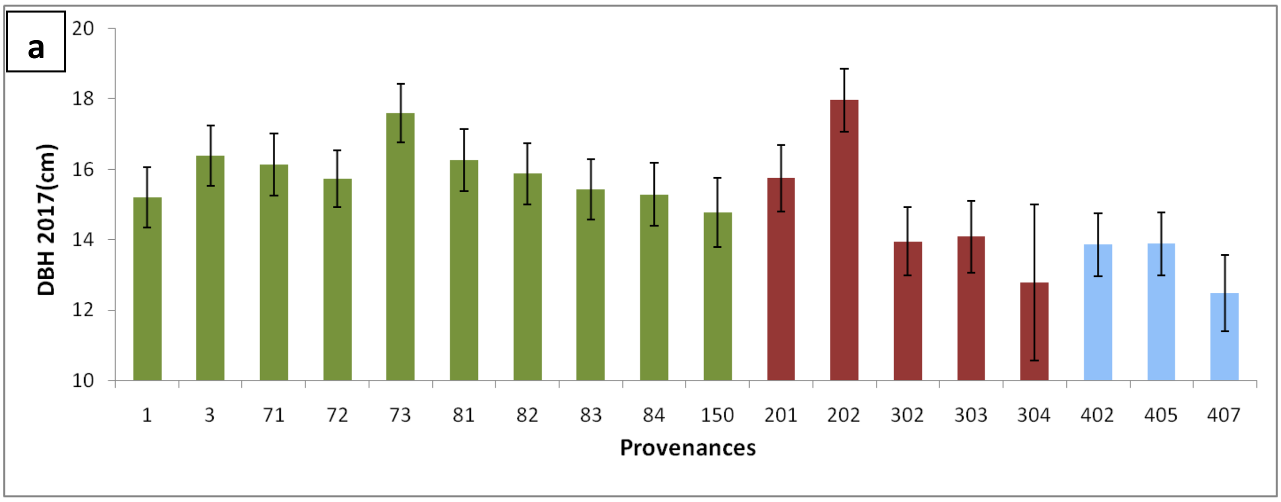


Figure 7: Residuals distribution of sugar (a) and starch (b) along with plantation row and column.

4.1.3. Diameter at breast height (DBH) and growth rate in relation with provenances and sub-species

In general DBH varied both in provenances (Figure 8a) and sub-species (Figure 8b) category; however, growth rate did not vary in any of those levels (Table 8), whereas it varied among different block (Table 10).



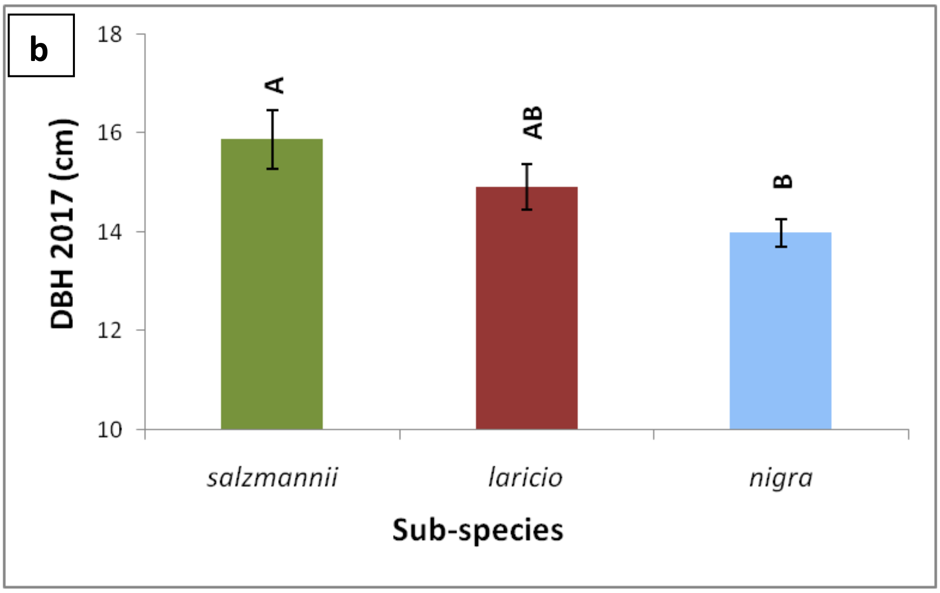


Figure 8: Least squares mean of DBH (with standard error bar) in different provenances (a) and sub-species (b).

Table 7: Linear mixed effect model of DBH; fixed effect: provenance, sub-species, provenance [sub-species] and block, whereas random effect variables: provenance* block, Tree [provenance, block].

Fixed Effect	**DBH (2017)**			
	df	df den	F Ratio	Prob > F
Provenance	17	77.7	2.11	0.01*
Sub-species	2	85.64	7.77	0.000*
Provenance[sub-spp]	15	77.11	1.42	0.160
Block	5	77.1	0.94	0.46

Random Effect	**REML Var Compnt, DBH (2017)**			
	Var ratio	Var compnt	Std Error	% of total
Provenance*Block	0.08	0.71	0.92	7.49
Tree[Provenance, Block]	-	8.60	1.15	92.51
Total	-	9.31	1.00	100

P values < 0.05 are marked by asterisk

Table 8: Linear mixed effect model of growth rate (2016-17); fixed effect: provenance, sub-species, provenance [sub-species] and block, whereas, random effect variables: provenance* block, Tree [provenance, block].

Fixed Effect	**Growth rate (2016-17)**			
	df	df den	F Ratio	Prob > F
Provenance	17	71.48	1.25	0.25
Sub-species	2	78.82	0.49	0.61
Provenance[Sub-spp]	15	70.90	1.24	0.26
Block	5	71.72	2.57	0.03*

Random Effect	**REML Var Compnt, Growth rate (2016-17)**			
	Var ratio	Var compnt	Std Error	% of total
Provenance*Block	0.13	0.01	0.02	7.24
Tree[Provenance, Block]	0.67	0.06	0.07	37.16
Residual	-	0.09	0.07	55.61
Total	-	0.17	0.01	100

P values < 0.05 are marked by asterisk

Provenances, as well as, sub-species of *P. nigra* exhibited significant ($P < 0.05$) differences of DBH in common garden condition, whereas, provenances under each sub-species category were not varied significantly ($P>0.05$) (Table 7). Only 7% of total variability of DBH was explained by provenances and block interaction, whereas almost 93% of total variability of DBH was explained by difference of individual trees within each provenances and blocks.

Mean separation test (student's T test) indicates that provenance 202 (Italian origin) has significantly higher DBH than provenance 1, 83, 84, 150, 302, 303, 304, 402, 405 and 407 of different origins (Table 9 and SI Table 3), whereas sub-species *salzmannii* and *laricio* have significantly higher DBH than sub-species *nigra.*

However, growth rate (2016-17) did not varied significantly ($P>0.05$) both in provenances and sub-species category, whereas, significant ($P<0.05$) difference of growth rate observed in different block (Table 11). Mean separation test revealed that block (III) have significantly higher growth rate than block (IV) (Table 11).

Nevertheless, there was no significant correlation ($P>0.05$, r = 0.13) between DBH and percentage of starch on different provenances of *P. nigra*.

Table 9: Least square mean difference (Student's T test) of DBH at provenances level, common or similar letter indicates no significant differences.

Provenance code	DBH Ranking	LS Mean
202	A	17.97
73	AB	17.59
3	ABC	16.38
81	ABCD	16.27
71	ABCD	16.14
82	ABCD	15.88
201	ABCD	15.74
72	ABCD	15.73
83	BCD	15.43
84	BCD	15.29
1	BCD	15.21
150	CDE	14.76
303	CDE	14.07
302	CDE	13.95
405	CDE	13.88
402	DE	13.85
304	BCDE	12.75
407	E	12.47

Table 10: Least square mean difference (student's T test) of DBH at sub-species level, common or similar letter indicates no significant differences.

Sub-species	**DBH**	
	Ranking	LS Mean
salzmannii	A	15.87
laricio	AB	14.89
nigra	B	13.41

Table 11: Least square mean difference (Student's T test) of growth rate (2016-17) in different blocks, common or similar lettering indicates no significant differences.

Block	**Growth rate (2016-17)**	
	Ranking	LS Mean
III	A	0.19
II	AB	0.18
I	ABC	0.17
VI	BC	0.15
V	BC	0.14
IV	C	0.14

4.1.4. Growth and Carbon investment according to climate and geographic origin of provenances

Carbon investment and DBH variability among different provenances and sub-species are controlled by several factors of climatic, geographical origin and also the combine effects of provenances and sub-species category.

Clustering of provenances on the basis of similar climatic origin, correlation of NSC and DBH with climatic variables as well as latitudinal origin of provenance and finally, Principle Coordinate Analysis (PCoA) and clustering was performed to visualize the combined influences of those variables on provenances and sub-species. It is to be noted that, the climatic condition of test site during sampling year (2017) was drier in comparison to 30 years average (1986-2016) shown in (Figure 3).

4.1.4.1. Clustering of provenances on the basis of similar climatic origin

Hierarchical clustering using climatic parameters in the origin of provenances (T_{max}, T_{min}, T_{range} and P_{annual}) generates three main provenance clusters (Figure: 9) having more or less similar climatic condition (Mean R^2: 0.736).

Within first and third cluster different sub-species are mixed together indicating more or less overlapping climatic conditions at the origin of different sub-species. In first cluster (red color) all sub-species of different provenance origin are grouped together; *salzmannii* (1, 3, 150), *laricio* (302, 303, 304, 201, 202), *nigra* (407), second cluster (green color) only sub-species *nigra* (402) and in third cluster (blue color) both sub-species *salzmannii* (71, 73, 82, 83, 81, 84, 72) and *nigra* (405) are present. It is to be noted that, first cluster's provenances are originating in more humid conditions (about 68%) and less mean T_{max} (about 5°C) and T_{range}(about 7°C) than third cluster's provenances, whereas second cluster's provenance have exceptionally high precipitation and low temperature than first and third clusters.

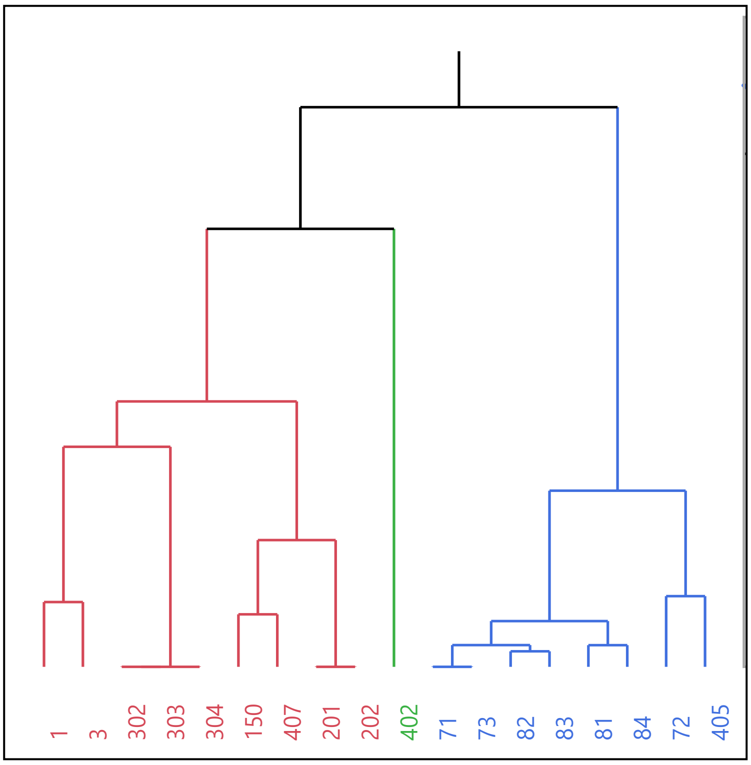


Figure 9: Hierarchical clustering of provenances using climatic variables (T_{max}, T_{min}, T_{range} and P_{annual}).

4.1.4.2. Correlations of climatic parameters in relation to NSC investment and DBH

From Table 12, it is clear that, percentage of total NSC, sugar and starch are significant ($P<0.05$) as well as positively correlated with T_{max} and T_{range} and negatively correlated with

T_{min} and P_{annual}; it is to be noted that, only sugar has significant ($P<0.05$) negative correlation with P_{annual}. More or less similar value of simple and partial correlations with NSC (total NSC, sugar and starch) and climatic parameters, indicating an actual association of those variables with both T_{max} and T_{range}. In contrast, the correlation of DBH and climatic variables are not strong enough (not significant), in every case the value of simple correlation is greater than partial correlation; indicating sub-species effects in explaining the correlation between DBH and climatic variables. That's mean depending on similar geographical and climatic origin different sub-species group together on the basis of DBH.

Table 12: Simple and partial correlation of total NSC, sugar, starch and climatic variables.

	T_{max}		T_{min}		T_{range}		P_{annual}	
	Simple Correlation	Partial Correlation	Simple Correlation	Partial Correlation	Simple Correlation	Partial Correlation	Simple Correlation	Partial Correlation
Total % NSC	0.48*	0.47	-0.28	-0.28	0.71***	0.75	-0.28	-0.26
% Sugar	0.57**	0.47	-0.17	-0.18	0.73***	0.67	-0.54*	-0.41
% Starch	0.48*	0.51	-0.28	-0.28	0.72***	0.78	-0.21	-0.18
DBH 2017	0.42 ($P = 0.08$)	0.21	0.08	0.11	0.38	0.12	-0.41 ($P = 0.08$)	-0.04

Significant correlation are indicated by (*) asterisks

4.1.4.3. Effect of latitudinal origin of provenances on NSC investment and DBH

In general, it is observed that provenances that are originating in lower latitude invest more NSC in branches during mid-July in comparison with provenance originating in higher latitude, however, only the soluble sugar concentration in branches has significant ($P<0.05$) correlation with latitudinal origin. Whereas, DBH also follow the same correlation like % of sugar; that means those provenances are originating in lower latitude have significantly ($P<0.05$) higher DBH than provenances origination in higher latitude (Figure 10).

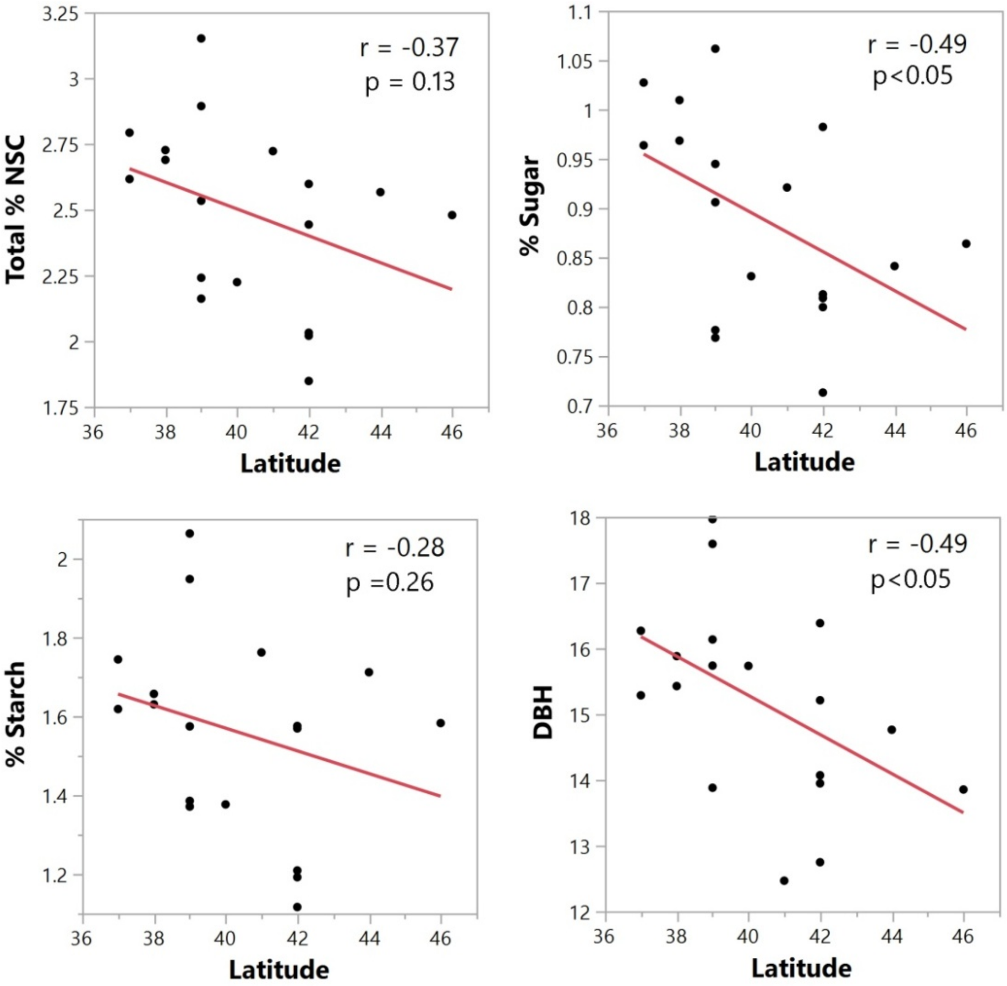


Figure 10: Relationship between NSC and DBH with latitudinal origin of provenances.

4.1.4.4. Environmental stress of different provenances in test site

As our experimental plantation site is located within the distribution range of *P. nigra,* so provenances in the test site have to be adapted with mainly three types of environmental conditions; (i) higher temperature or precipitation (ii) more or less same temperature and precipitation and (iii) lower temperature or precipitation, in relation to the origin of provenances.

The provenances in the left side of blue line are originated in relatively lower T_{max}, T_{min}, T_{range} and P_{annual} than the test site, however, the scenario is completely opposite in the right side of the blue line, that means those provenances are originated in relatively higher T_{max}, T_{min}, T_{range} and P_{annual} but now they are facing lower T_{max}, T_{min}, T_{range} and P_{annual}, whereas provenances intersected by blue line have similar climatic conditions in test site in accordance to their origin (Figure 11).

It is observed that those provenances originating in a conditions with lower T_{max} and T_{range} than the test plantation site have significantly ($P<0.05$) less % of sugar, starch reserve than the provenances originating in same or higher T_{max} and T_{range}; whereas T_{min} and P_{annual} have no particular correlations with starch, and sugar (except P_{annual} has significant negative correlation with sugar).

Furthermore, it is observed that DBH is less influenced by climatic parameters (T_{min}, T_{range} and P_{annual}), however, T_{max} have nearly significant correlation ($P = 0.09$) with DBH. In general, it is observed that those provenances originating in conditions with lower T_{max} and higher P_{annual} than the test plantation site have relatively less DBH (with few exceptions) than the provenances originating in same or higher T_{max} and P_{annual}, indicating unfavorable temperature and precipitation of test site reduce the DBH of provenances (Figure 11).

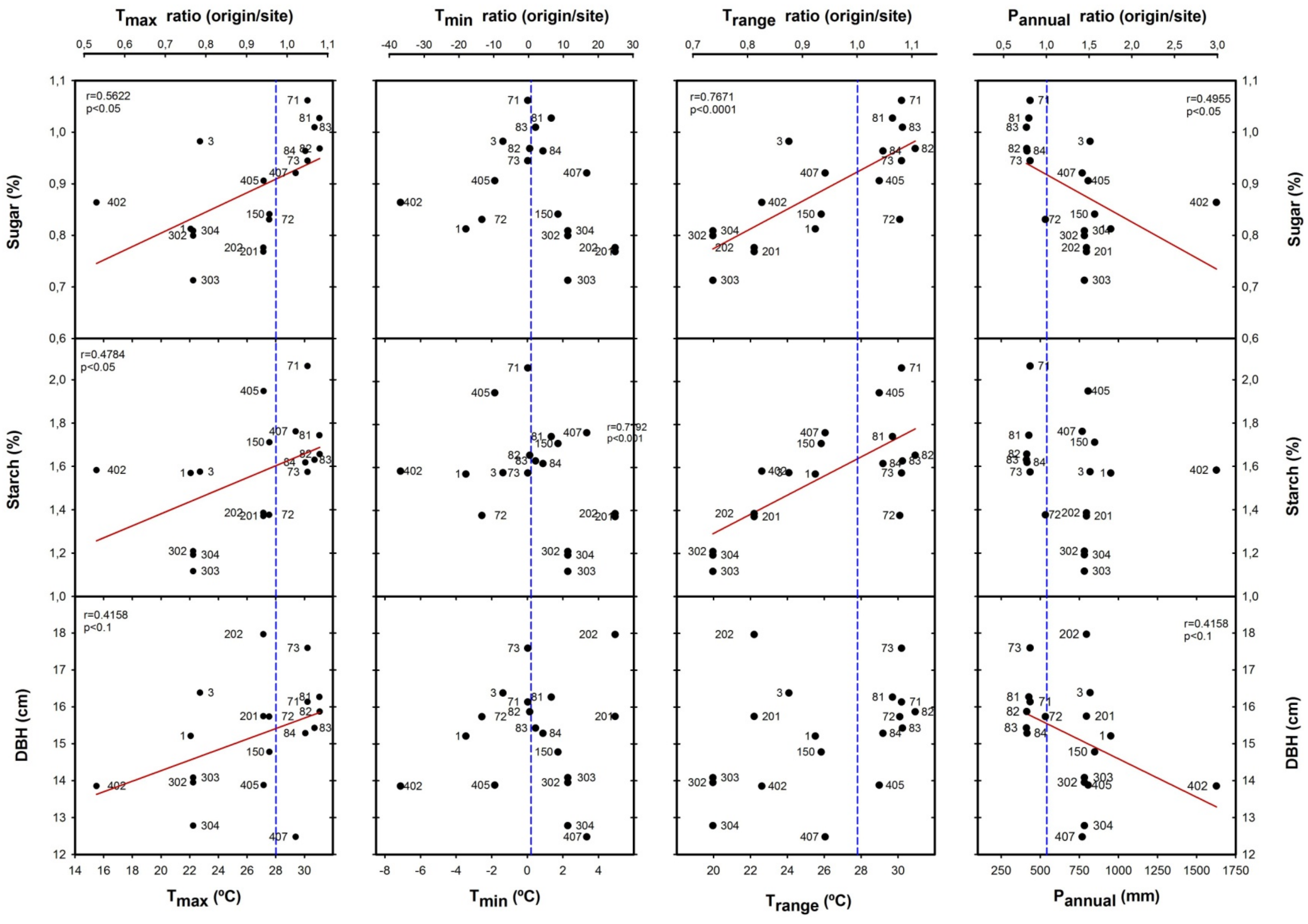


Figure 11: Relationship between NSC and DBH with Climatic variables of provenance origin. Blue line indicates the ratio of climatic condition of origin and test site and black filled circle indicates the provenances and red line indicates the correlation among variables.

4.1.4.5. Combined influence of NSC, DBH and climatic variables on provenances and sub-species

Depending on Principal Coordinate Analysis (PCoA) (Figure 12), 18 populations of *P. nigra* are dividing into four major groups (Figure 13) depending on basis of similarities in NSC investment, climatic conditions and DBH. It is to be noted that, the variability explains by PC-1 is almost 51.41%, whereas, PC-2 explain 17.98%, and in total these two axes explain 69.39 % of total variability. In general it is observed that % of sugar, starch and total NSC is positively associated with T_{range} and T_{max}, whereas negatively associated with P_{annual} and latitude. However, T_{min} have less influence on NSC accumulation in branch sapwood, whereas DBH have positive association with T_{max} and negative with latitude and P_{annual} (Figure 13).

Provenances under different sub-species category are group together on the basis of combined effect of NSC, DBH and climatic variables.

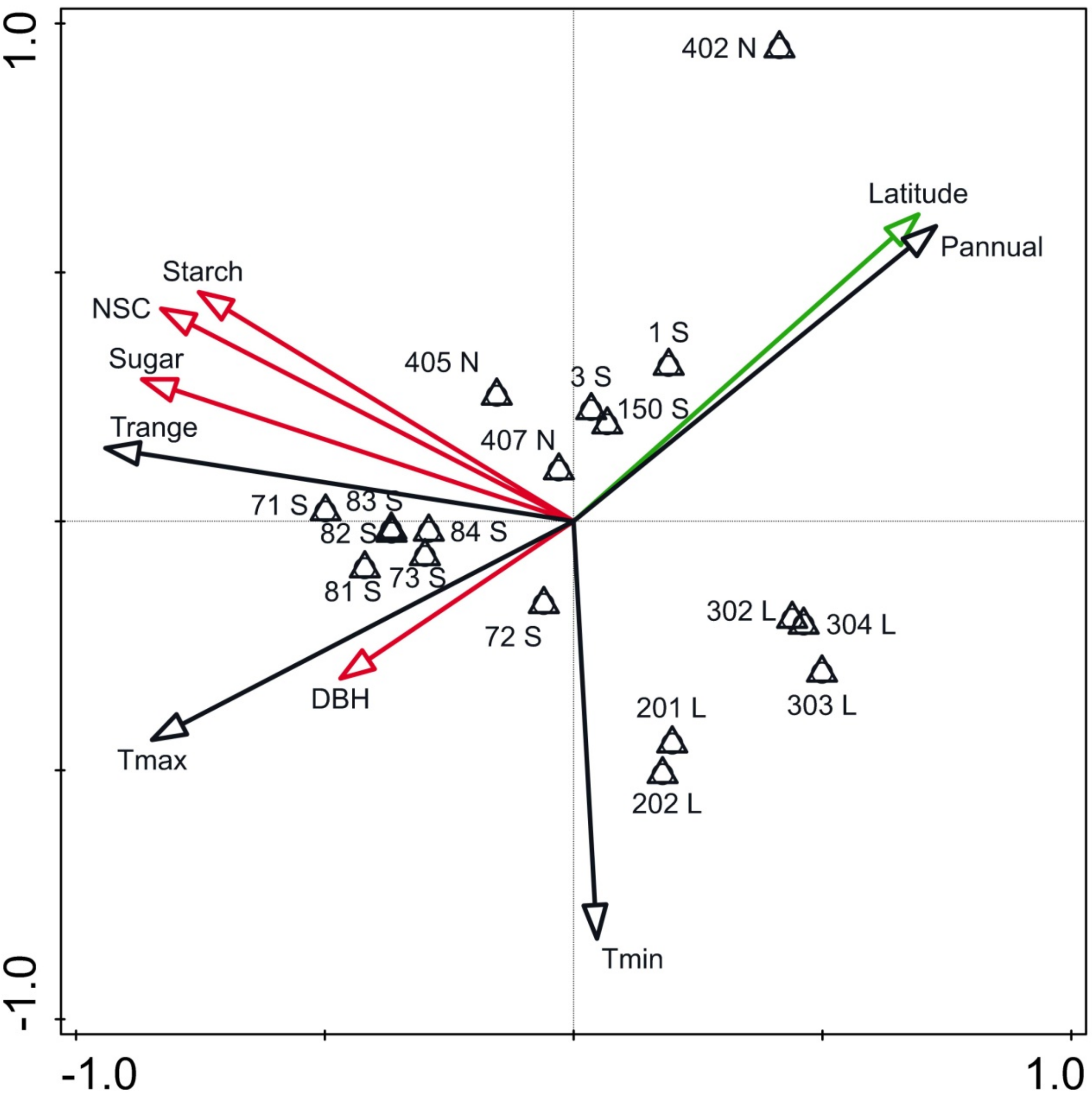


Figure 12: Principal coordinates analysis (PCoA) of all the variables (nonstructural carbohydrates, DBH, climatic parameters, provenance and sub-species).

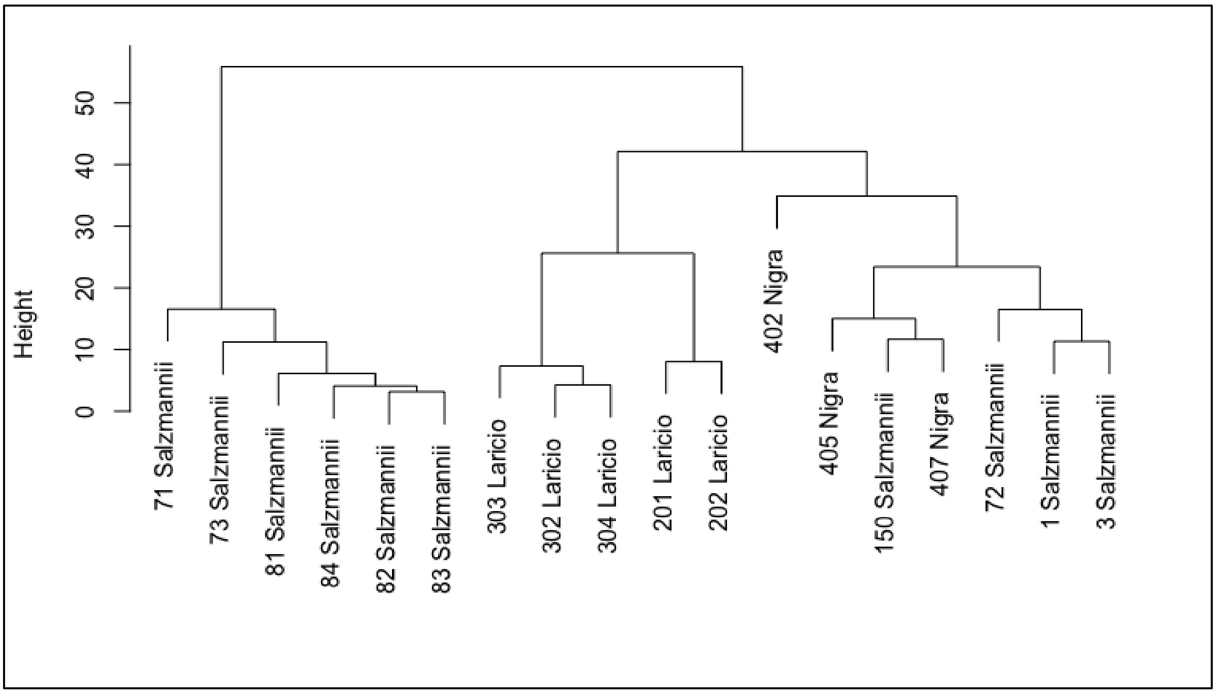


Figure 13: Cluster dendrogram of provenances by using climatic variables, NSC investment, and DBH

It is observed by comparing two hierarchical clustering (Figure 9 and Figure 13) that though different provenances/sub-species are originating in more or less similar climatic conditions (Figure 9) but according to their carbon storage investment and DBH they are more or less grouped by sub-species (Figure 13) that mean, similar climatic origin of provenances not only determine the diference in carbon storage and growth but there is also an effect of sub-species.

4.2. Discussion

4.2.1. NSC and DBH of provenances

Mediterranean black pine is one of the least studied conifer species. Its wide distribution area and genetic diversity and drought tolerance capability highlights its significance in climate change scenario. Common garden plantation of different provenances and sub-species of *P. nigra* in Segovia, Spain facilitates to test the carbon resource investment (in branch sapwood) and DBH variability among different provenances and sub-species level, as well as, to test the environmental stress in common garden conditions in accordance with geographic origin of provenances.

The findings of the study suggest that total non-structural carbon storage investment as well soluble portion (sugar) in branch sapwood in mid of July varied significantly only in sub-species but not at provenance level. On the other hand the non-soluble portion of stored carbohydrates (starch) varied both provenance and sub-species level, however; it cannot explain the significant variability of DBH in provenance level. Findings of the study also suggests that climate variables (mainly T_{max}, T_{min}, T_{range} and P_{annual}) on the geographic origin of provenances have influence in NSC accumulation in branch sapwood in mid-July, whereas DBH may have been slightly influenced by T_{max} and P_{annual} as well as climatic and geographic origin of provenances. In relation to stress in common garden environmental condition, it can be said that provenances originated in low T_{max} and T_{range} have lower NSC investment in comparison with provenances originating at similar or high T_{max} and T_{range}. However P_{annual} have negative relation only with soluble sugar investment that means, provenances originated in lower precipitation regime invest significantly higher soluble sugar in branch in mid-July, in comparison with provenances originating at similar or high P_{annual}. DBH is less influenced by climatic parameters (T_{min} and T_{range}), however, T_{max} and P_{annual} have nearly significant ($P = 0.1$) positive and negative association respectively with DBH.

Average concentration of starch was 57% higher than of soluble sugar in branch sapwood independent of provenances and sub-species category in the mid of July, may be because branches in top position are more self-sufficient in maintain reserve to cope with short-term imbalance between carbon supply and demand during and after the growing season (Plavcová and Jansen, 2015).

The significant difference of starch in provenance and sub-species level in common garden condition may be due to their genotypic responses of seasonal carbohydrates accumulation which most probably controlled by the temperature and geographic origin of the provenances. Though, there are no many studies on genetic variation in seasonal carbohydrates pattern; Oleksyn et al. (2000), reported seasonal accumulation and depletion of starch (in needle) in common garden condition of *Pinus sylvestris* was mainly mediated by temperature and geographic origin of provenances; in the same study they also reported that the concentrations of starch in needles and twigs were remarkably similar at all times.

In this study, heterogeneity of individual tree within each provenance and block was higher than the heterogeneity between provenances and sub-species level. Total NSC investment variability explain by the difference between 18 provenances is about 11% whereas; difference of individual trees within provenances is about 70%. Rubio-Moraga et al. (2012) measure the genetic variability amongst the *Pinus nigra* population of Spanish and Moroccan origin by using inter-simple sequence repeat markers and they found that variability explained by population is about 9% whereas, 52% was due to the difference among individuals within the population. Nikolić and Tucić (1983) also found the similar result with *P. nigra* and they explained the possible cause either by genetic drift or the local environments heterogeneity where gene frequencies are controlled through selection. Hamrick and Godt (1989) describe long life of gymnosperm maintain an intra-population genetic diversity because of high outcrossing rate and fecundity.

According to the result of this experiment, average concentration of branch sugar was about 0.91 % and starch was about 1.6 % in mid-July. NSC concentration of our study was relatively lower than the findings of other studies. Hoch et al. (2003) reported almost same amount of sugar and starch concentration in July (i.e., 3%) with the branch of *Pinus sylvestris*, however Oleksyn et al. (2000) found higher amount of starch (5.8%) and sugar (2%) in branched (bark + wood) of *Pinus sylvestris* in July. Piper et al. (2017) reported about NSC concentrations in dry and mesic site during mid-summer for *Pinus ponderosa* (sugar 6%, starch 1.5% and sugar 4%, starch 1.4% respectively) and *Pinus contorta* (about sugar 2.5%, starch 1% and sugar 2%, starch 0.8% respectively).

In this study, it was observed that the concentration of soluble sugar independently of provenance remains more or less the same indicating the metabolic importance of maintaining a minimum concentration of soluble sugars for respiration, vascular integrity and damage repair due to stress (Plavcová and Jansen, 2015). In common garden condition of *Pinus sylvestris* Oleksyn et al. (2000) also found no significant provenance effect on carbohydrates concentration in twigs (wood + bark).

Significant differences of DBH in provenance and at sub-species level indicate that some provenances as well as sub-species are comparatively more adapted to common garden climatic and soil conditions than the others. Varelides et al. (2001) reported that soil and climatic conditions have influence on growth differences (height and diameter) in a provenance trail of *P. nigra*. They also reported that provenances growth differences are more likely to appear in optimal condition. Delpierre et al. (2016) recently reported that the inter-annual variability of wood growth is driven by water stress due to drought.

Within the same sub-species, DBH varied significantly in different provenances (in case of *salzmannii* and *laricio*); in this relation, Piper et al. (2017) demonstrate local adaptation to stress is an important physiological trait among populations which actually determine the plant responses in diversified stress conditions. For instance, three populations of *Pinus ponderosa* seedling with different drought tolerance showed different growth sensitivity in response to water stress in controlled environment (Zhang et al. 1997).

No significant correlation between NSC concentration in branch and DBH, occur may be because NSC concentration in branch does not necessarily corresponding to the NSC concentration of stem (Hoch et al. 2003). On the other hand, (Jenkins et al. 1977) described photoperiodic response and timing of branch elongation (spring) and stem increment (autumn) are not the same, signifying their different timing of carbon allocation for growth. In another study (Klein et al. 2014) found that significant differences of tree's diameter growth under two different drought stress level was not reflected by the branch NSC level in *Pinus halepensis.*

4.2.2. Functional responses of genotypes to mild stress

Results of this study in common garden condition also indicate that provenances that are originating in lower latitude (37º N - 39º N) have 14% more starch and 11% more sugar in branch wood than provenances originating in higher latitude (40º N - 46º N) and branch soluble sugar is significantly associated with latitudinal origin. Oleksyn et al. (2000) also found the same association between latitudinal origin and needle soluble carbohydrates of *Pinus sylvestris* in common garden condition. It also observed that provenances those are originating in comparatively higher T_{max} regime than the common garden condition have about 28% more starch and 19% more soluble sugar reserve than those provenances originating in comparatively lower T_{max}. This relation is also true for T_{range}. As the latitude has a direct influence on temperature and precipitation so, may be climatic variables in the origin of provenance are the major determining factors for variation in carbon investment according to latitude.

It is also observed that provenances originated in lower precipitation regime than common garden have about 16% more sugar, 12% more starch concentration in branches and about 10% more DBH than the provenance originating in higher precipitation regime than common garden. It may be happen that, increased precipitation and in common garden have an influence on increased DBH as well as NSC reserve formation.

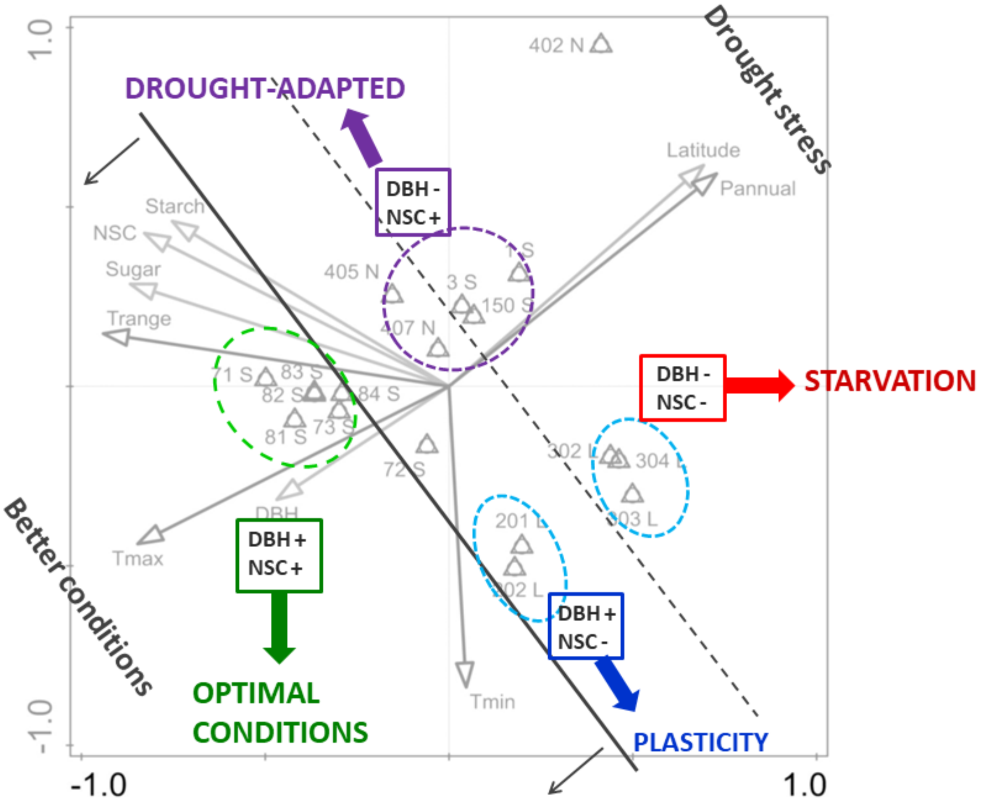


Figure 14. Genotypic functional behavior under contrasting climate changes scenarios.

On the other hand provenances originated in comparatively higher precipitation than common garden condition are subjected to mild stress and their NSC concentration and DBH both are reduced, which may be as result of carbon starvation where carbon reserve impeding growth which likely to be amplified in warmer and drier climate (McDowell et al 2011).

There are another two groups of provenances, one group (sub-species *nigra*, Greek and Italian origin, and *salzmannii*, French and Spain origins) reduces DBH and increased NSC, whereas another group (mainly sub-species *laricio*, Corsica, France origin) decreases NSC and increases DBH in response to reduced precipitation in common garden condition than their origin. First group perfectly follow the responses to drought stress trees described by Piper et al. (2017) signifying their ability to survive in upcoming climate change scenario. Furthermore, second group showed a very plastic behavior in maintaining high DBH in varieties of environmental conditions which was also reported by Varelides et al. (2001) in provenance trail of *Pinus nigra* in northern Greece..

Chapter 5: Conclusion

In summary it is observed that starch was the only source of variation among 18 provenances of *P. nigra* in branch NSC accumulation during mid-July, whereas both soluble sugar and starch displayed distinct difference in three sub-species category. Distinct difference of DBH also observed both in provenance and sub-species levels, however, current growth rate were not differed at all.

Differences in NSC accumulation (in branches) and DBH of provenances in test plantation site partly regulated by heredity and growth phenology on the basis of their climatic and geographic origin, as well as, their phenotypic response to environmental stress in test site, these are actually control the source and sink relationship and define the intra-specific variability in nonstructural carbon investment and DBH among provenances.

How to continue…..

To get a clear idea of intra-specific differentiation in carbon resource investment and to catch up the total variation of carbon dynamics it is recommended to operate sampling in different time period (according to the phenology of species) as well as organ level (leaf, stem and root as well).

It is also important to take into consideration other important tree and plant physiological variables, as well as, climatic and edaphic variables those have an influence on carbon resource investment.

After inclusion of Growth Rate on PCoA we detect a possible problem of competition. DBH and Growth Rate are separated in Factor II (Figure 15).

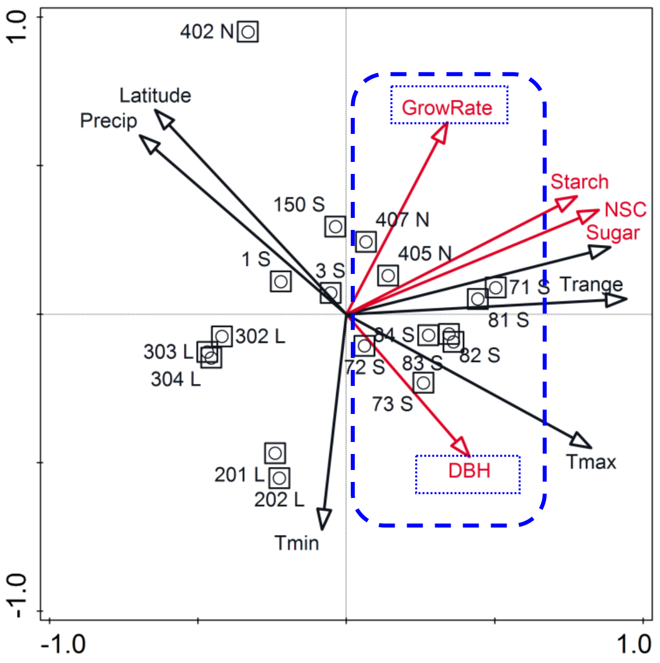


Figure 15. Principal coordinates analysis (PCoA) of all the variables (nonstructural carbohydrates, DBH, climatic parameters, provenance and sub-species), including Growth rate.

Another source of variation of sugar and starch concentration is due to the using different laboratory techniques. It is recommended to use a common laboratory technique to facilitate comparison.

Chapter 6: Bibliography

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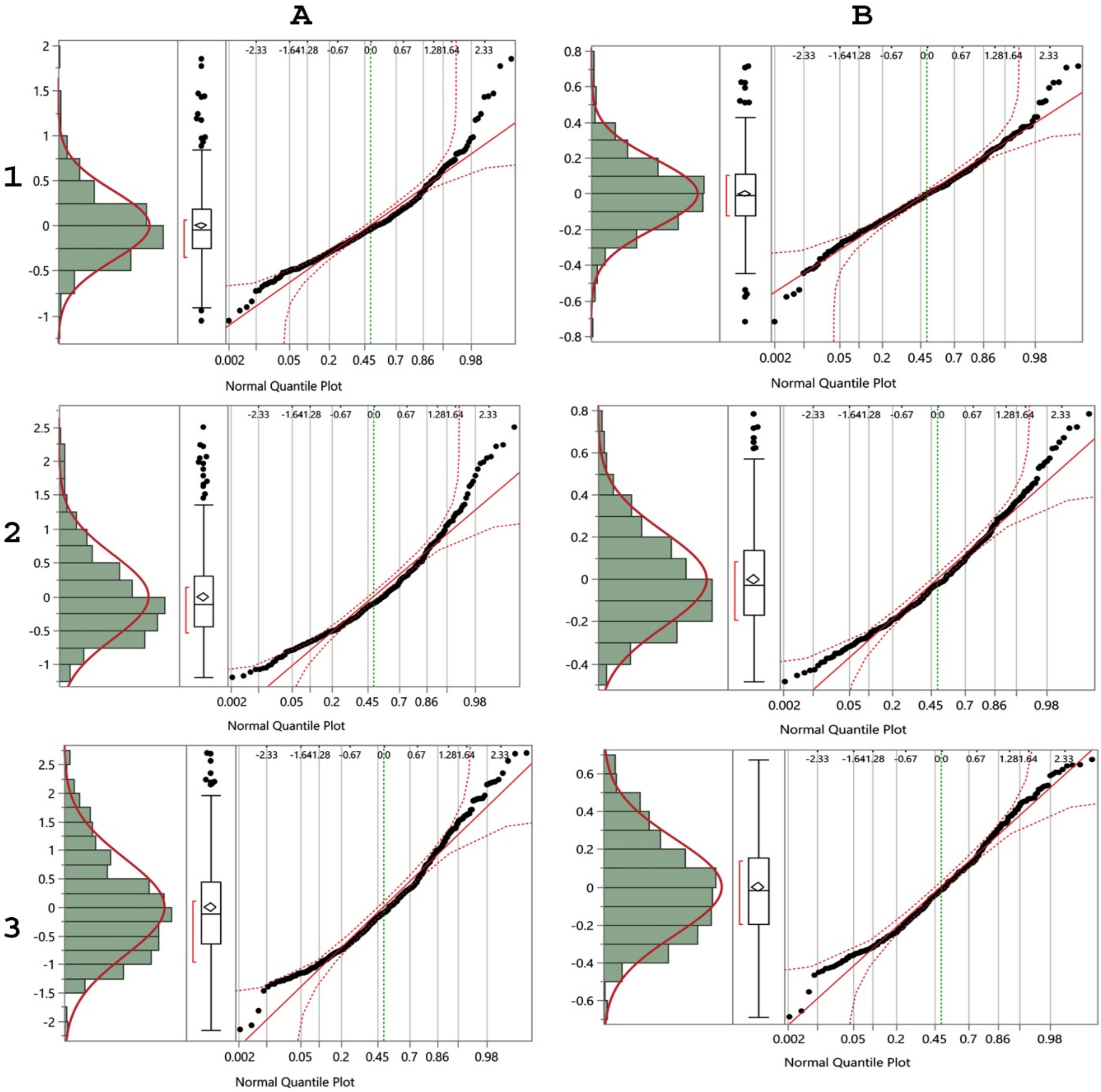
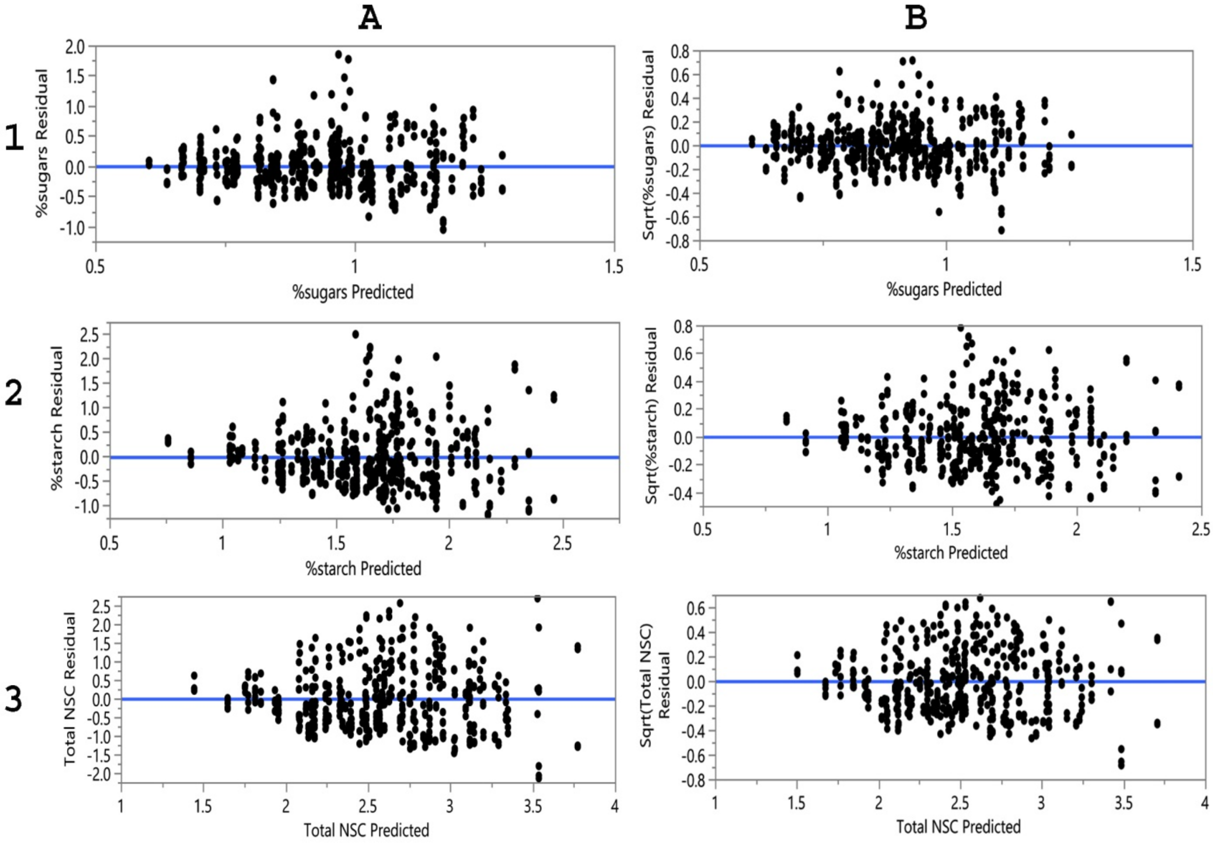
Supplementary information



SI Figure 1: Data normality before (A) and after (B) transformation of % Sugar (1), % Starch (2) and % NSC (3).



SI Figure 2: Residuals VS Predicted plot of before (A) and after (B) square root transformation of % Sugar (1) % Starch (2) % Total NSC (3)

Provenance Code	Sub-species	Country	Name	LS Mean Soluble Sugar %	LS Mean Starch %	LS Mean Total Sugar NSC%	LS Mean DBH 2017 (Cm)
1	*salzmannii*	ES	Ena	0.813	1.57	2.44	15.21
3	*salzmannii*	ES	Solsona	0.98	1.58	2.60	16.38
71	*salzmannii*	ES	Cuenca	1.06	2.06	3.15	16.14
72	*salzmannii*	ES	Los Palancares	0.83	1.38	2.22	15.74
73	*salzmannii*	ES	Los Cadorzos	0.95	1.57	2.53	17.60
81	*salzmannii*	ES	Navahondona	1.03	1.74	2.79	16.27
82	*salzmannii*	ES	Paterna del Madera	0.97	1.66	2.69	15.87
83	*salzmannii*	ES	Cazorla-Alcaraz	1.01	1.63	2.73	15.43
84	*salzmannii*	ES	Huescar	0.96	1.62	2.62	15.29
150	*salzmannii*	FR	Gagnieres	0.84	1.71	2.57	14.78
201	*laricio*	IT	Grancia	0.77	1.37	2.16	15.75
202	*laricio*	IT	MachiadellaTavolla	0.78	1.39	2.24	17.97
302	*laricio*	FR	Noceta	0.81	1.21	2.03	13.95
303	*laricio*	FR	Sorba	0.71	1.12	1.85	14.08
304	*laricio*	FR	Ghisoni	0.81	1.19	2.02	12.78
402	*nigra*	AU	Parabluberg	0.86	1.58	2.48	13.85
405	*nigra*	GR	Milea	0.91	1.95	2.90	13.88
407	*nigra*	IT	Villeta	0.92	1.76	2.72	12.48

SI Table 3: Mean concentration of percentage soluble sugar, starch, total NSC and DBH according to provenance