1 **Original Article**

2 **Rhizome trait scaling relationships are modulated by growth conditions and are linked**

3 **to plant fitness**

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8 **AUTHOR CONTRIBUTIONS:**

9 ZM owned the original data used in this study. DT and ZM conceived the idea and

10 conceptualised the study. DT analysed the data with help from ZM. DT wrote the draft of the

11 paper with in☐depth suggestions, inputs and editing from ZM. Both the authors approved the

12 final version of the manuscript.

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22   **ABSTRACT**

23   *Background and Aim:* Rhizomes are important organs allowing many clonal plants to persist

24   and reproduce under stressful climates with longer rhizomes indicating enhanced ability of

25   the plants to spread vegetatively. We do not however, know either how rhizome construction

26   costs change with increasing length or vary with environmental conditions.

27   *Methods:* We analysed the rhizome length vs mass scaling relationship, the plasticity in the

28   scaling relationships, their genetic basis, and how scaling relationships are linked to plant

29   fitness. We used data from 275 genotypes of a clonal grass *Festuca rubra* originating from 11

30   localities and cultivated under four contrasting climates. Data were analysed using standard

31   major axis regression, mixed-effect regression models and a structural equation model.

32   *Key Results:* Rhizome construction costs increased (i.e., lower specific rhizome length) with

33   increasing length. The trait scaling relationships were modulated by cultivation climate and

34   its effects also interacted with the climate of origin of the experimental plants. With

35   increasing length, increasing moisture led to a greater increase in rhizome construction costs.

36   Plants with lower rhizome construction costs showed significantly higher fitness.

37   *Conclusions:* This study suggests that rhizome scaling relationships are plastic, but also show

38   genetic differentiation and are linked to plant fitness. Therefore, to persist under variable

39   environments modulation in scaling relationships could be an important plants' strategy.

40   **Key words:** Allometry; Clonal grass; Climate change; Plasticity; Resource allocation

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INTRODUCTION

Plants optimise the allocation of limited resources to different plant parts to increase growth, survival and reproduction in a given environment (Reich et al., 1997; Wright et al., 2004; Díaz et al., 2016). Changes in environmental conditions may thus modify the allocation patterns via resource trade-offs, which, eventually, affect allocation to organs representing fitness, e.g., reproductive organs or total biomass (Springate and Kover, 2014; Halbritter et al., 2018). Elucidating the patterns of allocation and determining factors that cause changes in resource allocation patterns are, therefore, critical for understanding plant carbon economics and, hence, plant functioning and persistence (long term survival) under current and future climates.

To explore allocation strategies and developing plant economic spectra, trait scaling relationships are very important (Wright et al., 2004; Niklas et al., 2007; Enquist et al., 2015). In the last two decades, the use of these relationships in ecological research has greatly enhanced our understanding of how resources are allocated in different plant parts, such as, leaves, twigs or stems (Niklas et al., 2007; Fajardo, 2016; Husáková et al., 2018; Deng et al., 2020). The basic expectation behind the scaling relationships is that the various traits are not independent but, rather, are linked to other traits via trade-offs (Reich 2014). Based on the scaling relationships among various plant traits, a range of studies have demonstrated that allocation to some plant functions increases with increasing size (e.g., increased leaf construction cost for larger leaves (Niklas et al., 2007); increased investment into the stem relative to the leaves for larger trees (Poorter et al., 2015)). This indicates that plants allocate disproportionately more resources into specific plant functions with changes in size (Poorter et al., 2015).

Most studies on trait scaling relationships in plants deal with leaf traits (e.g., Reich et al., 1997, Wright et al., 2004, Niklas et al., 2007, Milla and Reich, 2007), with fewer studies

67 dealing with stem traits (Poorter et al., 2015), root traits (Chen et al., 2019; Deng et al., 2020)

68 and plant biomass fractions (above-ground and below-ground) (Niklas and Enquist, 2001;

69 Husáková et al., 2018). There is, however, no such information for rhizomes and Goldberg et

70 al., (2020) recently highlighted the strong need to understand the allocation of resources to

71 clonal growth in plants.

72   Rhizomes are ecologically important plant organs responsible for vegetative spread in

73 many plant species (Klimešová and De Bello, 2009; Duchoslavová and Jansa, 2018), with

74 increased importance in stressful climates, for example, above the tree-line (Billings and

75 Mooney, 1968; Körner, 2003). Rhizomes form bud banks (Ott et al., 2019) and enable

76 species persistence by tolerating extreme conditions (e.g. very low or high temperatures or

77 flooding) and promoting rapid growth during favourable seasons (since they also act as

78 storage organs) (Billings and Mooney, 1968; Hull, 2008; Kinmonth-Schultz and Kim, 2011).

79 Rhizomes also have important roles in processes such as below-ground carbon sequestration

80 (De Deyn et al., 2008), community assembly (Weiher et al., 1998) and plant community

81 resilience to disturbances (Hull, 2008; Speed et al., 2010).

82   The length of the rhizome per unit dry mass investment (i.e., specific rhizome length

83 (SR_zL = rhizome length / dry mass)) is a key trait indicative of costs of vegetative spread.

84 Specific rhizome length was shown to vary with nutrient availability (Ye et al., 2006) and

85 rhizome burial and breakage (Balestri and Lardicci 2014). Based on patterns reported in the

86 literature on scaling relationships among above-ground traits (e.g., Reich et al., 1997, Wright

87 et al., 2004, Niklas et al., 2007, Milla and Reich, 2007), the construction costs of rhizomes

88 with increasing length can increase, decrease or be independent of their length. We are,

89 however, not aware of any study distinguishing between these scenarios. Such knowledge is

90 nonetheless important and has implications in understanding the ability of clonal plants to

91 spread vegetatively and grow.

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92   Climate is a major driver of plant functioning with vast literature on patterns, causes

93   and consequences of functional trait variation along climatic gradients. By contrast, little is

94   known about the effects of climate on trait scaling relationships (Vasseur et al., 2018).

95   Specifically, earlier studies analysing variation in scaling relationships due to climatic factors

96   (e.g., Xiang et al., 2013; Fajardo, 2016; Klimešová et al., 2017; Thakur et al., 2019) have not

97   separated the effects of specific environmental factors on these relationships. This might be

98   because the majority of the earlier studies was carried out in very complex natural

99   environments where multiple factors (e.g., soil properties, climate, and genetic changes) act

100   at the same time. We thus need experimental studies to disentangle the role of each factor

101   separately.

102   In addition to allowing the separation of the effects of single environmental factors,

103   the great advantage of experimental studies is that they allow assessment of the specific

104   mechanisms determining the scaling relationships. Specifically, by studying plants from

105   different original conditions cultivated under different environmental conditions, it is possible

106   to assess the relative importance of genetic differentiation and phenotypic plasticity in

107   determining the scaling relationships. Genetic differentiation has a role in the generation and

108   maintenance of biodiversity and, thereby, insight into the evolutionary processes and the

109   adaptive divergence of populations may be. gained (Weiher et al., 1998). On the other hand,

110   phenotypic plasticity allows individual organisms to develop the appropriate traits that are

111   better suited to the particular environment that they encounter (Yan et al., 2013). While

112   studies separating these effects in terms of plant performance are common (e.g.,

113   (Münzbergová et al., 2017; Datta et al., 2017; Manzanedo et al., 2019), studies doing so in

114   terms of trait scaling relationships are rare (but see Vasseur et al., 2018)).

115   Changes in trait values such as in specific root length (Kramer-Walter et al., 2016),

116   specific leaf area (Liu et al., 2016) and rhizome density (Meyer and Schmid, 1999) have been

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117   shown to affect carbon allocation and thus be linked to whole plant fitness. Similarly,

118   Vasseur et al., (2018) demonstrated that changes in trait scaling relationships may also lead to

119   changes in carbon use and thus to changes in plant fitness. The effects of scaling relationships

120   on fitness may also depend on environmental conditions determining the resource use

121   efficiency of the plants (Anderson, 2016; Mota et al., 2018). Our knowledge on the effects of

122   the scaling relationships on fitness and its changes within a changing environment is,

123   however, still very limited.

124   To fill the above-mentioned gaps in our knowledge, we aim to understand rhizome

125   length vs mass scaling relationships, in general, in order to test the effect of climate (the

126   original and cultivation, representing genetic differentiation and phenotypic plasticity) on

127   these   relationships and explore how these relationships are related to plant fitness. Using

128   *Festuca rubra* as a model plant, we addressed the following questions: (1) How do rhizome

129   length and mass scale with each other? (2) Do trait relationships change with changes in

130   temperature and moisture of growth conditions and plant origin? (3) Are rhizome trait scaling

131   relationships linked to plant fitness?

132   We hypothesise that as with leaf trait scaling relationships, the rhizome length will

133   fail to keep pace with increasing mass. We expect that the scaling relationships will vary with

134   growth conditions, as a result of which the plants growing in warmer and wetter climates

135   should have a lower rate of increase in tissue construction costs (because there is no thermal

136   or water limitation). We also hypothesise that the trait relationships differ between plants

137   from different original climates. Additionally, it is expected that lower rhizome construction

138   cost in longer rhizomes would lead to higher plant fitness.

139   **MATERIALS AND METHODS**

140   *Study species and system*

141   The data used in this study are from Münzbergová et al., (2017) on a widespread

142   clonal grass *Festuca rubra* L. It is distributed in temperate to tundra regions in both

143   hemispheres as well as in tropical mountains (GBIF.org 2021, accessed on 07/03/2021). It

144   reproduces vegetatively by forming both intra-vaginal and extra-vaginal tillers on rhizomes,

145   but also reproduces by seeds (Münzbergová et al., 2017). While reproducing vegetatively,

146   this species varies in the growth form and, consequently, resource capture strategies, creating

147   a continuum of forms between 'phalanx' and 'guerilla' strategy (Skálová et al., 1997).

148   Opposite to the 'guerilla' strategy, the 'phalanx' strategy is characterised by a decrease in the

149   length of rhizome internodes and a greater rate of branching (Lopez et al., 1994). The

150   'phalanx' strategy is adopted in resource-rich environments while the 'guerilla' strategy is

151   favoured in resource-poor conditions (Skálová et al., 1997).

152   The dataset used represented trait values of 275 genotypes (the genotype

153   identification method is given in Šurinová et al,. 2019) collected from 11 localities (25

154   genotypes per locality). These localities are part of a climatic grid (the SeedClim Grid) of

155   factorially-crossed temperature and precipitation gradients in western Norway (see details in

156   Klanderud et al., 2015). All these genotypes were cultivated in four growth chambers (Votch,

157   2014), simulating the climatic conditions of extreme localities of the climate grid (cold/warm

158   combined with dry/wet, (with circles in Figure 1) resulting in 11 localities $\times$ 25 genotypes $\times$ 4

159   climates, (i.e., 1100 individuals) were grown in the experiment. The conditions in the growth

160   chambers mimic the spring to summer climate of each population in the field (the second half

161   of April to the second half of June). Prior to cultivation, the plants were grown in a common

162   climate for about seven months in total (four months in the garden and three months in the

163   greenhouse) to remove most of the transgenerational effects (Münzbergová et al., 2017; see

164   also Münzbergová et al., 2019).

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166   *Growth conditions*

167   The temperature in each the growth chambers followed the course of temperatures at

168   the simulated natural locality (for details, see Münzbergová et al., 2017). The moisture level

169   in the growth chambers was monitored as soil moisture using TMS dataloggers (Wild et al.,

170   2019) (three inside each chamber) and then adding the necessary amount of water to mimic

171   the soil moisture of the natural localities (also monitored using the TMS dataloggers). Each

172   data-logger was placed in a pot with a growing *Festuca* plant identical to the experimental

173   plants, but which was not a part of the experiment (not used for any other measurements). In

174   the climate chambers with dry conditions, plants were watered with about 20 mL of tap water

175   per plant, applied to the trays if the soil moisture was lower than 15%. In the wet regime,

176   plants were cultivated under full soil saturation with ~1.5 cm water level in the tray. By

177   manipulating the soil moisture in the growth chambers, we are mimicking soil moisture at

178   localities with a certain precipitation level. We thus refer to the moisture conditions in the

179   growth chamber as precipitation values of the simulated localities. Day length and radiation

180   levels were also controlled in a manner that these mimic the conditions of the original

181   localities (more details in Münzbergová et al., 2017).

182   Some studies suggest that performing experiments in growth chambers lead to

183   pseudo-replication of the experiment (Hurlbert, 1984). This argumentation has been later

184   refuted as non-justified (e.g., Oksanen, 2001; Johnson et al., 2016). Most importantly, even

185   Hurlbert (2004) stated that such a setting is not a problem in cases when the main focus is not

186   on the main effects of the chamber climate, but on any interactive effects, such as the rhizome

187   scaling relationships in our case (see (Münzbergová et al., 2017) for an extended discussion

188   of the issue).

189

190   *Plant cultivation*

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191   The experiment ran from the end of February to the end of August 2015. The

192   experiment was initiated by planting a single ramet without any rhizomes and with roots

193   developed in water over three weeks prior to planting into each pot. Thanks to this, all below-

194   ground structures of the experimental plants were of identical age.

195   In mid-June 2015, all the above-ground parts were cut to 3 cm to simulate mowing in

196   natural conditions. After the final harvest in August 2015, we separated the plants into above-

197   ground and below-ground parts; the below-ground parts were carefully sorted into roots and

198   rhizomes. The total length of all rhizomes per plant was measured and all the biomass was

199   then dried to a constant mass at 60 °C and weighed. The number of rhizomes per plant could

200   not be counted due to their possible breaking during their extraction from the soil, so we only

201   have information on their total length. This restricts us from mentioning if the total rhizome

202   length is due to one rhizome or multiple rhizomes. This does not, however, have any

203   influence on the results as, in any case, (i.e., the total length is due to one or more rhizomes)

204   the overall longer length of the rhizome(s) per plant would reflect a greater ability to spread.

205

206   *Plant traits*

207   This study primarily focused on analysing the relationship between rhizome length

208   (mm) and rhizome mass (g). In addition, we also used other measured traits from the same

209   individuals (i.e., above-ground biomass (g), number of ramets, total plant biomass (g), net

210   photosynthetic rate (PN) and specific leaf area (SLA, mm^2/mg) estimated by Münzbergová et

211   al., (2017), Stojanova et al., (2018) and Kosová et al., (2021) to test if plant fitness and

212   performance are linked to changes in the scaling relationships. Since this species reproduces

213   predominantly clonally and only rarely flowered during the course of the study

214   (Münzbergová et al., 2017), we used above-ground biomass, number of ramets and total plant

215   biomass as estimates of plant fitness. Biomass is one of the widely used estimate of plant

216   fitness (Younginger et al., 2017) and number of ramets is considered as good estimate of

217   fitness in grasses (Pan and Price 2001). We also linked the scaling relationships to net

218   photosynthetic rate (PN) and specific leaf area (SLA) to understand how changes in rhizome

219   mass per unit length with increasing length is linked to changes in leaf ability to fix carbon

220   and leaf construction cost. Due to the high workload required to measure these two traits, the

221   data on these are only available from 10 genotypes originating from each of the four most

222   extreme climates of the climatic grid: wettest and driest combined with warmest and coldest,

223   i.e., 4 original climates × 4 cultivation climates × 10 genotypes = 160 measurements in total

224   (the same as in Kosová et al., 2021).

225

226   ***Data analyses***

227   *Standardised major axis regression*

228   Standardised major axis (SMA) regression, a method commonly used for analysing

229   scaling relationships, was used to calculate scaling exponent and elevation (α and β,

230   respectively) for the relationship between rhizome length and mass (Niklas et al., 2007;

231   Warton et al., 2012). SMA regression is most appropriate when both variables have errors

232   and there is not a clear distinction between dependent and independent variables (Warton et

233   al., 2012). A large number of earlier studies had used SMA to explore change in dry mass

234   investment with increasing leaf area. It has been described using the formulae:

235    $Area = \beta Mass^{\alpha}$, where β is the elevation and α is the scaling exponent of the log-transformed

236   area vs. mass regression curve (Niklas et al., 2007). In the case of rhizomes, the formulae will

237   take the form $Length = \beta_1 Mass^{\alpha}$. Since $SR_zL = Length/Mass$ and $Mass = \beta_2 Length^{\alpha}$, it

238   follows that $SR_zL = (1/\beta_2)\ Length^{1-\alpha}$. The value of α>1 in this equation would indicate that

239   length fails to keep pace with mass (diminishing returns), whereas α<1 means the opposite

240   (increasing returns).

241   Using the SMA regression, first, we estimated the scaling exponents and elevation for

242 each cultivation climate across all populations in order to identify how the general scaling

243 exponent is modulated by the cultivation climate. Second, we separately estimated the scaling

244 exponents for plants from each original climate (population) grown at each cultivation

245 climate to test if trait scaling relationships of plants from different original climates differ

246 when grown in similar climate. The same analysis will also permit testing if the scaling

247 exponents for plants from the same climate of origin differ when cultivated in different

248 climates. In all the cases, we tested if the value of the scaling exponent is significantly

249 different from 1 using one sample t-test. Using multiple post-hoc comparisons, we tested for

250 differences in scaling exponents between plants grown in different cultivation climates and

251 among plants from different original climates grown in different cultivation climates. SMA

252 was done using 'sma' function with the argument 'multcomp=TRUE' (for multiple

253 comparisons) in the 'SMATR' package (Warton et al., 2012) of R Version 4.0.3 (R Core

254 Team and Core R Team, 2019).

255   Using SMA regression we also explored the effect of the direction of climate change

256 on scaling relationships. For this, we estimated the change in temperature (ChangeT) and the

257 change in moisture (ChangeM) by subtracting cultivation climate values from the original

258 climate (expressed as °C for temperature and mm of rainfall, see above). For instance, if the

259 original temperature is 6.5°C and the cultivation temperature is 12.5°C, the temperature

260 change is +6°C (for more details see Münzbergová et al., 2017). We then estimated the

261 scaling exponents based on differences in moisture or temperature between the original and

262 the cultivation climate. Since scaling exponents differed significantly based on changes in the

263 moisture, we also tested how change in moisture affects scaling exponents (direction of

264 effect) using linear regression ('lm' function in R).

265 *Mixed-effect models*

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266   While SMA regression is the best method to analyse the trait relationships, it does not

267   allow study of the effects of different factors and their interactions in these relationships.

268   Further, it does not allow consideration of any additional structure of the data (in our case,

269   genotypes). We thus also analysed the data using mixed-effects regression models (see

270   Husáková et al., 2018 for a similar approach). In the model, we used length as a dependent

271   variable and mass as an explanatory variable, with original climate (temperature and

272   precipitation, referred to as Otemp and Omois respectively), cultivation climate (referred to

273   as Ttemp and Tmois) and interactions as fixed factors, and genotype as a random factor.

274   Since the model was complex (with up to five interacting factors) we used the Akaike

275   Information Criterion (AIC) to select the best model (Säfken et al,. 2018).

276   We also used the mixed effects regression to explore the effect of the direction of

277   climate change on trait relationships, providing a more straightforward interpretation of the

278   possible interactions between the climate of origin and that of cultivation. For this, we used

279   ChangeT and ChangeM as fixed factors in the model and also tested their interactions with

280   mass. As above, genotype was used as a random factor. Mixed-effects regression was carried

281   n out using 'lmer' function in 'lme4' package (Bates et al., 2015) of R Version 4.0.3 (R Core

282   Team and Core R Team 2019). Rhizome length and mass values were log-transformed before

283   model fitting.

284   *Relationships with fitness*

285   To get an overview of the effect of the scaling exponent on plant fitness and resource

286   capture traits, we used structural equation modelling (SEM) using the 'sem' function of the

287   'lavaan' package (Rosseel, 2012) of R. Data points in SEM were values of the scaling

288   exponent, one for each population and cultivation conditions, and average trait values based

289   on the same plants; one also for each population and cultivation conditions. In the SEM, we

290   only used the variables that were significantly related to the scaling exponent. The

291 relationship was tested using linear regression ('lm' function in R), (for details see

292 supplementary information S1). We considered three possible measures of plant fitness in the

293 SEM, the number of ramets and above-ground biomass and the total biomass. As the scaling

294 exponent was not related to the number of ramets and above-ground biomass, only total

295 biomass was used in the SEM presented. While doing the SEM, the best model was selected

296 based on the lowest AIC value of the model and highest p value of Chi-Square (χ^2)

297 (Schermelleh-Engel et al., 2003; Barrett, 2007). The other measures of goodness of model fit

298 (comparative fit index, Tucker Lewis index, root mean square error of approximation, and the

299 standardised root mean square residual) as recommended by Coughlan et al., (2008) were

300 also used to determine the model fit.

301 In SEM, we used the scaling exponent as the explanatory variable for response

302 variables (rhizome length, SLA, and total biomass). We also used SLA as explanatory

303 variable for PN and rhizome length. PN was also used as an explanatory variable for rhizome

304 length. SLA and rhizome length were also used as explanatory variables for total biomass

305 (indicator of fitness). Additionally, we also ran the model by using biomass excluding

306 rhizomes and total biomass as indicators of fitness; the results were largely similar (not

307 shown).

308

309 **RESULTS**

310 *Effects of the cultivation climate on the scaling relationships*

311 The mixed-effects regression results revealed that the relationship between rhizome length

312 and mass was affected by both the moisture and the temperature of the cultivation climate

313 (i.e., driven by phenotypic plasticity); the effect of moisture was, however, stronger (Table

314 1). The effects of temperature and moisture did not, however, interact (Table 1).

315   From the SMA regression, we found that plants cultivated in different cultivation

316   climates differed significantly in the numerical values of α, see Table 2. The value of the

317   scaling exponent (α) was significantly greater than 1 in all four cultivation climates (Table 2

318   and Figure 2). This indicates that rhizome dry mass increases disproportionately faster than

319   rhizome length. In other words, rhizome construction costs increase with increasing length.

320   As a consequence, longer rhizomes tend to have lower SR_zL. The value of the scaling

321   exponent (α) was significantly larger in plants cultivated in wet climates (both warm wet and

322   cold wet) than in dry climates (Table 2 and Figure 2). This indicates that larger rhizomes are

323   even more costly (in terms of biomass investment per unit length) in wet than in dry

324   cultivation climates.

325   Despite significant differences in the scaling exponents among the four cultivation

326   climates, the value of the scaling exponents was always significantly greater than 1 (Figure

327   2). This means that across various cultivation climates, the increases in rhizome mass result

328   in disproportionately less gains in rhizome length (because α > 1,) but the gain is even lower

329   in wet climates. When the value of the scaling exponents from each climate of origin in each

330   cultivation climate were tested against the value of 1, it was found that the value of the

331   scaling exponent was either equal to 1 or greater than 1 but was never less than 1. This

332   indicated that there could be a proportionate increase in rhizome length and mass or there is a

333   disproportionately higher increase in mass than length, but length can never increase

334   proportionately greater than mass.

335

336   ***Effects of the climate of origin on the scaling relationships***

337   In the linear mixed-effects regression analysis, there were no significant interactions

338   of mass with original climate only (Table 1). The only significant effects of the original

339   climate were in interactions with the cultivation climate (i.e., Mass:Tmois:Otemp,

340   Mass:Ttemp:Otemp, Mass:Tmois:Ttemp:Otemp in Table 1) indicating that plasticity in the

341   relationships between rhizome length and rhizome mass depends upon the climate of origin.

342   Additionally, in the SMA results, there were significant differences among plants from

343   different original climates within a cultivation climate (evident only in T3_M1 cultivation

344   climate -(see Table 3 and Figure 3) as well as between cultivation climates (e.g., T1_M1 and

345   T3_M4 in Table 3). Scaling exponents also differed in plants from the same climate of origin

346   when they were grown in different cultivation climates (e.g., in T2_M1 and T2_M4). The

347   value of the scaling exponent for each original climate across all the cultivation climates

348   ranged from 0.903 (statistically not different from 1; e.g., climate of origin T2_M4 grown in

349   warm and dry climate, T3_M1) to 1.395 (significantly greater than 1 (e.g., genotypes from

350   climate T3_M4 grown in cold and wet climate, T1_M4.

351

352   ***Effects of climate change on scaling relationships***

353   In the mixed-effects regression analysis based on degree of change in temperature and

354   moisture between the climate of origin and the cultivation climate, significant interaction

355   between 'Mass' and 'ChangeM' (change in moisture) was found (Table 4). This indicated

356   differing scaling exponents based on changes in moisture. The SMA regression also revealed

357   similar results and the value of the scaling exponent significantly increased with an increase

358   in moisture (Figure 4). Change in temperature had no effect on the scaling exponent. This

359   indicates that increasing or decreasing temperatures does not cause any additional change in

360   rhizome construction cost with increasing length, but, with an increase in moisture, the

361   rhizome mass (construction cost) increases disproportionately more than the rhizome length

362   at an increased rate.

363

364   ***Relationship between scaling relationships and plant fitness***

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365   The high p value of the χ^2 (0.99) as well as other estimated measures (e.g., Tucker

366 Lewis index, root mean square error of approximation etc.) indicated a good fit of the

367 structural equation model (supplementary Table S2.5). The model was able to moderately

368 explain the variances in each of the response variables (R^2 ranged from 0.34 to 0.68) (Figure

369 5). The model revealed that the increasing value of the scaling exponent significantly

370 negatively influenced rhizome length and SLA, indicating that both rhizome length and SLA

371 increases with decreasing value of the scaling exponent. The increasing SLA further led to an

372 enhanced photosynthetic rate (PN) thereby increasing the total carbon fixed by the plant, but

373 the PN was not significantly related to total biomass. When SLA were higher, total biomass

374 also tends to be higher and was evident in the model from a marginally-significant, positive

375 relationship (p=0.054) between the two. However, increasing SLA also led to decreasing

376 rhizome length. Additionally, longer rhizomes were also significantly positively linked with

377 higher plant total biomass.

378 **DISCUSSION**

379   Trait scaling relationships have important implications for understanding the plant

380 resource use strategies and the ability of plants to acclimatise or adapt to variable

381 environmental conditions. In this study, we analysed the rhizome length versus mass scaling

382 relationships and found a considerable variation in the scaling exponents. The main findings

383 emanating from this study are: (i) The rhizome length fails to keep pace with increasing mass

384 and, as a result, with increasing rhizome length the rhizome construction costs increase. (ii)

385 Trait scaling relationships are modulated by climatic conditions in which the plants grow, and

386 their effect interacts with the climate of origin. (iii) Climate change affects the scaling

387 relationships and wetter climates lead to greater increase in rhizome construction costs with

388 increasing length. (iv) Trait scaling relationships are closely linked to plant performance (e.g.,

389 carbon capture capacity by leaves).

390

Rhizome construction cost increases with increasing length

392   Our analyses show that scaling relationships for the functional traits that primarily

393   influence plant vegetative spread have a scaling exponent greater than one in all the

394   cultivation climates. Thus, changes in rhizome length fail to keep pace with increasing mass.

395   This indicates that either bulk rhizome-tissue density or thickness (or both) increase as

396   rhizome length increases (causing decreased SR_ZL). The result is similar to those reported for

397   above-ground plant traits (e.g., leaf size and leaf mass, Leishman et al., 2007; Price and

398   Enquist, 2007; Atkin et al., 2008)). Theoretically, these results mean diminishing returns

399   from an increase in rhizome length (i.e., increasing construction cost for larger rhizomes).

400   This finding is in parallel with Niklas et al., (2007) and (Milla and Reich 2007) who reported

401   'diminishing returns' in the case of leaves.

402   The finding follows the metabolic scaling theory (Enquist et al., 2007) supporting the

403   fact that trait relationships are similar across evolutionary-distinct organisms as well as in

404   ecologically distinct functional traits. We assume that this increasing cost could be because

405   rhizomes are also the organs to transport water and minerals to above-ground parts, additional

406   mechanical support is, therefore, required for longer distance transport. Additionally,

407   rhizomes are also storage organs in grasses (for soluble as well as for non-soluble

408   carbohydrates (Klimeš et al., 1999; Kinmonth-Schultz and Kim, 2011), but this feature is

409   unlikely to cause higher mass per unit length with increasing length. Although such

410   information is missing in the literature, we also do not have any reason to expect that storage

411   function of rhizomes would affect scaling between the rhizome mass and the length (i.e.,

412   larger rhizomes should store disproportionately more or less carbohydrates per unit length

413   than smaller ones). Nevertheless, testing if rhizome length and stored carbohydrates have

414   isometric relationship is an open question.

17

415   Further, the study species is commonly grazed in natural habitats, so longer rhizomes

416   are more vulnerable to herbivore damage via trampling. Therefore, greater investment in

417   mechanical tissues (more xylem or thick-walled cells) may also be helpful in this case. In this

418   context, Striker et al., (2006) stated that the porosity of roots in a grass species (*Paspalum*

419   *dilatatum*) is lower (density is higher) when exposed to trampling. Although there is

420   additional cost associated with longer rhizomes, longer rhizomes enable positioning of new

421   ramets away from the parents thereby decreasing intra-clone competition and increasing

422   resource availability and the higher investment is thus reasonable. This might be an important

423   strategy of clonal plants and may lead to overall positive returns (e.g., higher dominance in a

424   community).

425

426   ***Scaling relationships are modulated by growth conditions***

427   Rhizome length vs mass scaling relationships varied among plants cultivated under

428   different climatic conditions demonstrating that scaling relationships are modulated in

429   response to growth conditions. While there are no previous studies on rhizome scaling

430   relationships allowing comparison with our results, several previous studies showed the effect

431   of climate on scaling relationships of leaf traits (e.g., Wright et al., 2005; Atkin et al., 2008;

432   Xiang et al., 2013; Thakur et al., 2019). All these studies were, however, from natural

433   environments and were not able to separate phenotypic plasticity from genetic differentiation.

434   By using data from experimentally-manipulated conditions, we were able to separate multiple

435   effects in this study. Our results provide strong evidence that plant scaling relationships are

436   plastic. This finding is important because evidence of the plasticity in scaling exponents is

437   lacking, particularly in plants (for animals, see Casasa & Moczek, 2019)). Specifically, these

438   findings indicate the importance of modulation in scaling relationships under variable

439   climates in *Festuca rubra*. We argue that similar to the role of trait plasticity in plant

18

440   persistence under variable climates, plasticity in scaling relationships is also important and

441   might be helpful in optimal resource allocation under changing climatic conditions to

442   enhance plant fitness.

443   The variation in scaling relationships with changing cultivation conditions might help

444   the plant to balance the need for efficient vegetative spread, resource use and protection

445   against desiccation and soil herbivores. The direction of the shift in the scaling exponent

446   (lower value in drier sites) was, however, unexpected. The majority of the previous literature

447   indicates that warmer and wetter conditions favour plant growth (Natali et al., 2012;

448   Buermann et al., 2018) but our data indicate the opposite (because higher tissue construction

449   costs have negative consequences). This could be because the wet climate was too wet

450   (simulation of 2700 mm of annual rainfall) and thus not favourable for the species as also

451   claimed by Münzbergová et al., (2017). These too wet conditions may create hypoxic

452   conditions; it is known that plants in hypoxic conditions have thick, aerenchymatic below-

453   ground structures (Pedersen et al., 2021). To increase thickness there would be additional

454   allocation of resources in the aerenchyma (which otherwise is not needed) and when

455   rhizomes are longer, more thicker tissues can be helpful for longer-distance, oxygen

456   transport. Therefore, more carbon allocation is needed with increasing rhizome length.

457   Secondly, a transition towards a 'phalanx' resource use strategy (higher number of ramets per

458   unit length) was also evident in this species in wet climates (Supplementary Figure S2.1) and

459   this transition could be responsible for the higher rate of increase in rhizome construction cost

460   with increasing length. This can be expected because plants with 'phalanx' strategy have

461   shorter internodes, which are linked to increased stem density, and decreased hydraulic

462   conductivity (Jacobsen et al., 2020). The decrease in hydraulic conductivity with increasing

463   number of nodes is because some of the vessels end in each internode thus disrupting the

464   resource flow (Jacobsen et al., 2020). Therefore, with increasing rhizome length, the need to

465   maintain hydraulic conductivity of plants in wet conditions having many internodes may

466   impose additional costs. Third, with increasing wetness, there might be increased activity of

467   soil herbivores or parasitic fungi (Velásquez et al., 2018). Hence, additional investments per

468   unit length are needed for protection because tissues with lower carbon content are easier to

469   degrade (Silver and Miya, 2001). Overall, our results indicate that it is more economical for

470   this clonal species to form longer rhizomes in conditions with lower water availability. This

471   was evident in the results as plants in drier conditions had longer rhizomes (Supplementary

472   Figure S2.2).

473   The differences in the value of scaling exponents among cultivation climates were

474   apparently very small but, as shown by Milla & Reich (2007), the small differences in the

475   numerical value of the scaling exponent (among cultivation climates in our case) can translate

476   into very large differences in construction cost per unit length when rhizomes differ greatly in

477   length. These small differences can have huge consequences for the overall plant

478   performance (e.g., vegetative spread) because of greater cost to build and maintain a unit of

479   rhizome length, which should constrain the maximum rhizome length achieved by a plant.

480   Milla and Reich (2007) demonstrated that 1.07 value of the scaling exponent in leaf area vs

481   mass relationship cause 22% increase in per unit leaf construction cost, when leaf size

482   increased by about 6.5-fold (i.e., 96 to 622 cm^2). Considering this example, one can imagine

483   the impact of only a small deviation of the scaling exponent from 1.

484   Interestingly, in this study, the total length of the rhizome in climates with a greater

485   increase in construction cost (e.g., warm and wet) remained far less than in climates with a

486   lesser increase in construction cost (see Supplementary Figure S2.2). We argue that there

487   might be economic constraints that limit rhizome elongation (or ability to spread) when

488   construction cost is increasing rapidly. This shorter length of the rhizome in warm and wet

489   climates may, therefore, be the negative consequence of greater increase in construction cost.

490   Our result also hints that plants tend to keep the rhizome construction cost under a certain

491   threshold, above which rhizomes are not formed. This was supported by the fact that SR_zL

492   was not significantly lower in warm and wet cultivation climate than dry and wet climate, but

493   rhizome length was kept short. Moreover, despite shorter rhizomes, the SR_zL at the

494   cultivation climates with higher construction costs have higher variance (inferred based on

495   trait driver theory (Enquist et al., 2015), (Supplementary Figure S2.3). This indicated that in

496   such climates, plants are beginning to construct rhizomes with a lower construction cost but,

497   as a consequence of a larger value of the scaling exponent, construction cost surpassed to that

498   in drier climates at much shorter lengths.

499

500   *Genetic differentiation in the scaling relationship*

501   We suspected that scaling relationships might also be result of adaptation to

502   environments at the places of origin. While we did not find any significant interaction

503   between mass and the climate of origin, we detected triple interactions between mass, the

504   climate of origin and that of cultivation indicating genetic differentiation in the plasticity of

505   the scaling relationships. This finding provided supporting evidence to possible local

506   adaptation in scaling relationships associated with phenotypic optimisation to enhance

507   fitness. This is in line with Vasseur et al., (2018) providing evidence for a genetic basis in the

508   scaling relationship of plant dry mass vs growth rate in *Arabidopsis thaliana*.

509   In combination with the effect of climate change on the scaling relationships, our

510   results support the idea that scaling relationships are modulated significantly by original as

511   well as cultivation climate at intraspecific levels. Therefore, we suspect that, at community

512   level, differences in scaling relationships (reported in many earlier studies on leaf traits

513   (Niklas and Enquist, 2001; Milla and Reich, 2007; Niklas et al., 2007; Thakur et al., 2019))

21

514 could be result of both intraspecific (i.e., responses to climate and local adaptation) as well as

515 interspecific effects (i.e., due to species turnover).

516

517 *Scaling relationships are linked to plant fitness*

518 The inverse relationship of the scaling exponent with the total plant biomass indicated

519 that plant fitness decreases when formation of longer rhizomes becomes more costly (i.e., a

520 larger value of the scaling exponent). This was also expected because higher investment per

521 unit length can have negative consequences (less investment) for other traits involved in

522 resource acquisition. The SEM result supports that decreased scaling exponent was associated

523 with lower leaf construction cost (i.e. SLA). In turn, this lower leaf construction cost was

524 associated with higher photosynthetic efficiency. Therefore, when leaf construction costs are

525 lower, more light can be intercepted by the same aboveground mass (Milla and Reich 2007)

526 and, subsequently, more carbon is fixed. Overall, performance of leaves was greatly

527 increased when the scaling exponent was lower.

528 Our results also indicated that when the value of scaling exponent is lower, longer

529 rhizomes are formed indicating the plant's ability to vegetatively spread is also enhanced.

530 Additionally, the net returns as represented by total biomass were also dependent on rhizome

531 length (the greater the length, the higher the returns), (see also supplementary Figure S1.1).

532 The negative relationship between the SLA and rhizome length in the SEM indicates that the

533 'exploitative' resource use strategy of leaves is linked to the limited ability of plants to spread

534 vegetatively. The probable reason is that when leaves are more productive, more resources

535 are allocated to roots to exploit more nutrients from the soil. This was supported by a strong

536 positive relationship between SLA and root biomass (Supplementary figure S2.4).

537 Our results on the relationship of the scaling exponent with leaf traits (PN and SLA)

538 also indicate that changes in below-ground tissue construction costs are linked to above-

539   ground tissue construction costs. We could have tested this hypothesis in this study, but the

540   available data of leaf area and leaf mass did not allow us to do so (as the SLA was based on

541   data from leaf fragments). Overall, the findings of this study provided evidence that when

542   scaling relationships are modulated, plant fitness is compromised. The scaling exponents

543   indicating 'diminishing returns' (as defined in Niklas et al., 2007) had negative consequences

544   on plant fitness. As we have very scarce information about plant investments into rhizomes

545   (in comparison with other plant organs) and how these investments are modulated by growth

546   conditions, more such studies are awaited to know if the patterns are similar across clonal

547   species with different phylogenetic histories. While many current studies are dealing with the

548   benefits of clonal growth, more studies (such as this one) are needed to shed light on clonal

549   growth costs. It can be expected that as with above-ground plant organs, the patterns

550   observed in this study may also stand true for other species that reproduce mainly clonally.

551   Nevertheless, this needs to be tested in diverse species occurring in different ecoregions.

552

Conclusions

554   Our analysis of the scaling of rhizome length vs mass indicates that rhizome length

555   fails to scale one to one with rhizome mass due to which longer rhizomes have higher

556   construction costs (i.e., lower SR_zL). Our findings provided evidence for plasticity in scaling

557   relationships in plant traits and demonstrated that the rate of increase in rhizome construction

558   costs with length depends upon the growth conditions. Among the climatic conditions, the

559   moisture of the cultivation conditions was the main determinant of the scaling relationships.

560   Our results also provided additional support for genetic basis in the scaling relationship.

561   Finally, we have demonstrated that scaling relationships are closely linked to plant

562   performance and lower scaling exponents (with length, a lower increase in construction cost)

563   are linked to traits representing acquisitive resource use strategy.

23

564

CONFLICT OF INTEREST

The authors declare no conflict of interest

567

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760   of plant fitness? *Applications in Plant Sciences* **5**: 1600094.

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763 **Table 1.** Linear mixed-effects regression model describing the effect of rhizome mass (g),

764 original (O) and cultivation (T) climate (temperature and moisture) and their interactions on

765 rhizome length (mm). Genotype was used as a random factor in the model. Only effects of

766 mass alone and in interaction with the other factors are shown here. Full results of the model

767 are given in supplementary Table S2.1. Significant values ($p < 0.05$) are shown in bold.

Fixed factors	F value	P value
Mass	**30.67**	**<0.001**
Mass:Tmois	**9.37**	**0.002**
Mass:Ttemp	**4.66**	**0.031**
Mass:Omois	1.17	0.279
Mass:Otemp	2.53	0.112
Mass:Tmois:Ttemp	**4.65**	**0.031**
Mass:Tmois:Omois	2.80	0.095
Mass:Tmois:Otemp	**8.12**	**0.004**
Mass:Ttemp:Omois	3.29	0.070
Mass:Ttemp:Otemp	**5.03**	**0.025**
Mass:Omois:Otemp	0.42	0.518
Mass:Tmois:Ttemp:Omois	1.55	0.213
Mass:Tmois:Ttemp:Otemp	**6.45**	**0.011**
Mass:Tmois:Omois:Otemp	3.39	0.066
Mass:Ttemp:Omois:Otemp	1.17	0.280

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770 **Table 2.** Scaling exponent (α) and elevation (β) for scaling relationship between rhizome

771 length (mm) and rhizome mass (g) of *Festuca rubra*. The plants in different cultivation

772 climates differ among each other in their scaling relationships. The value of scaling

773 exponents in all the cases (presented in bold) were significantly greater than 1. 95%

774 confidence intervals of α and β are given in supplementary Table S2.2. The superscript letters

775 represent significant differences in scaling exponent among cultivation climates.

Cultivation climate	Scaling exponent (α)	Elevation (β)	R^2
Cold_Dry	**1.099** [a]	-2.768 [a]	0.925
Cold_Wet	**1.203**[b]	-2.904[b]	0.846
Warm_Dry	**1.042** [a]	-2.768 [a]	0.935
Warm_Wet	**1.217**[b]	-2.981[b]	0.849

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778 **Table 3.** Scaling exponent (α) and elevation (β) of scaling relationship between rhizome

779 length (mm) and rhizome mass (g) based on the origin climate and the cultivation climate. In

780 each case the value of R^2 was > 0.647. The values of scaling exponents in bold are

781 significantly greater than 1. 95% confidence intervals of α are given in supplementary Table

782 S2.3. ** represents that scaling exponents differs among original climates for plants grown in

783 the same cultivation climate (in the same column) and [##] represent that plants from the same

784 original climate differ in scaling exponent among different cultivation climates (in the same

785 row).

	Cultivation climate							
	Cold_Dry (T1_M1)		Cold_Wet (T1_M4)		Warm_Dry (T3_M1)**		Warm_Wet (T3_M4)	
Origin climate	α	β	α	β	α	β	α	β
T1_M1	1.082	-2.811	**1.385**	-2.993	**1.183**	-2.981	**1.310**	-3.063
T1_M3	0.997	-2.620	**1.201**	-2.906	**1.156**	-2.980	**1.265**	-2.994
T1_M4	0.967	-2.488	**1.221**	-2.952	1.005	-2.622	1.115	-2.861
T2_M1[##]	1.111	-2.771	**1.253**	-2.884	0.982	-2.652	**1.281**	-2.986
T2_M2	1.000	-2.626	1.099	-2.818	**1.177**	-2.951	1.214	-3.036
T2_M3	1.088	-2.793	**1.287**	-2.988	1.034	-2.774	**1.324**	-3.072
T2_M4[##]	**1.296**	-3.056	**1.254**	-2.988	0.903	-2.557	**1.266**	-3.081
T3_M1	1.101	-2.833	1.083	-2.785	0.999	-2.733	1.006	-2.734
T3_M2	1.107	-2.796	1.140	-2.893	0.957	-2.658	1.147	-2.944
T3_M3	1.085	-2.754	1.153	-2.814	1.085	-2.799	**1.199**	-3.090
T3_M4	1.102	-2.731	**1.395**	-3.064	0.986	-2.660	1.066	-2.647

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788 **Table 4:** Linear mixed-effects regression model statistics describing the effect of rhizome

789 mass (g), climate change (temperature and moisture) and their interactions on rhizome length

790 (mm). Genotype was used as a random factor. Only effects of mass alone and its interaction

791 with the other factors are shown here. Full results of the model are given in supplementary

792 Table 2.4. Significant values ($p < 0.05$) are shown in bold.

Fixed factors	F value	P value
Mass	**7853**	**< 0.001**
Mass:changeT	2.40	0.136
Mass:changeM	**20.51**	**<0.001**
Mass:changeT:changeM	3.83	0.059

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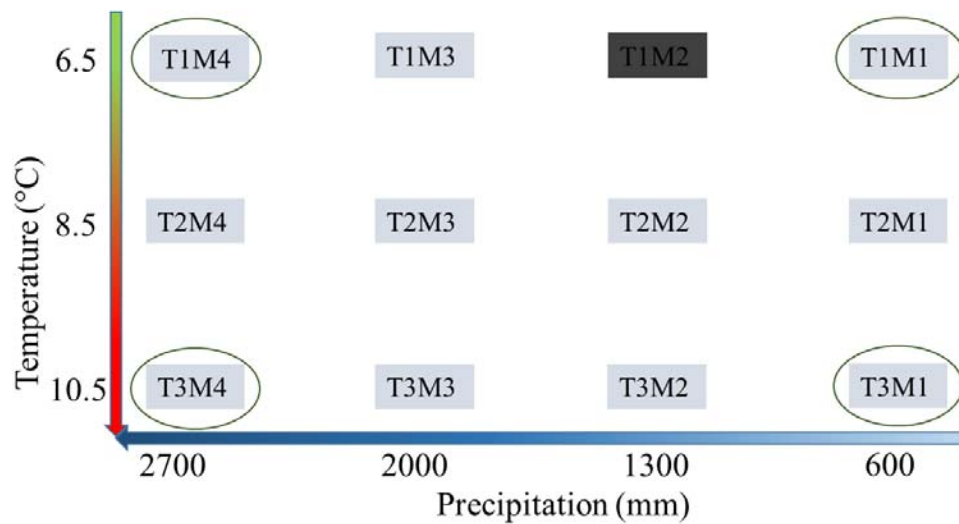
Figure 1: Representation of the climatic conditions of the localities from where the plant material was sampled. The four climates that are encircled were also used in climatic chambers as cultivation climates. The climatic condition in dark grey background (T1M2) was not used due to unavailability of the species. Temperature is mean for growing season and precipitation is annual.
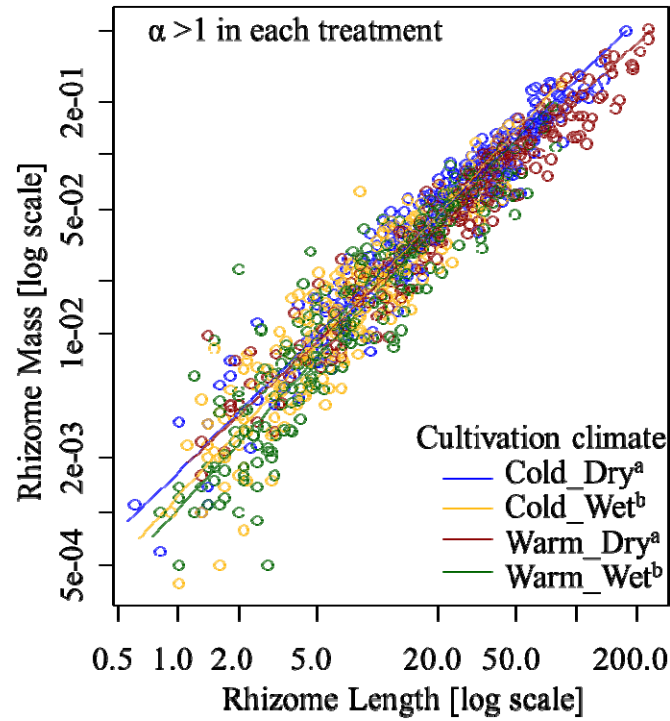
Figure 2: Relationship between rhizome length (mm) and mass (g) for plants from different cultivation climates. The superscript letters represent significant differences in scaling exponent among cultivation climates.
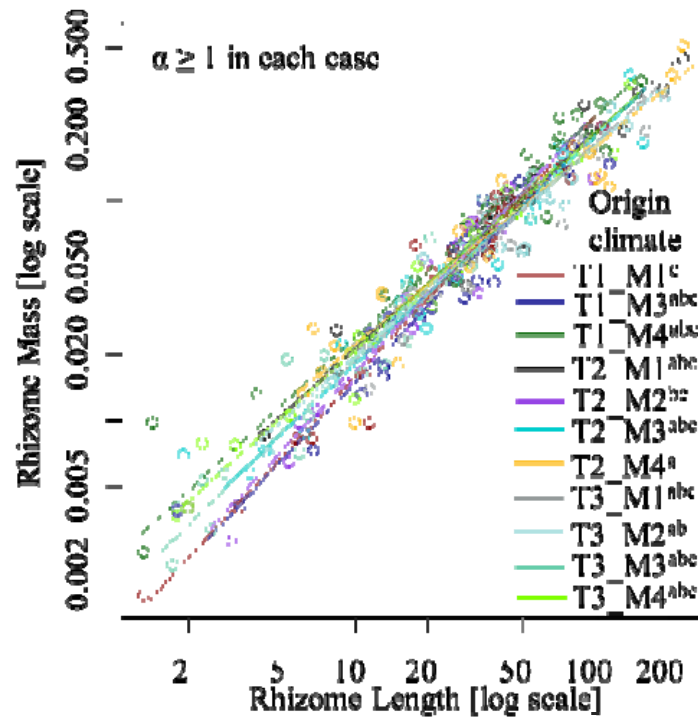
Figure 3: Differences in scaling exponent for the relationship between rhizome length (mm) and mass (g) among plants from different original climates grown under warm and dry cultivation climate. The plot is shown only for warm and dry cultivation climate because in other cultivation climates the scaling exponents were statistically not different among each other. T1 to T3 represent origin temperatures from low to high and M1 to M4 represent origin moistures from low to high (for details see Figure 1).
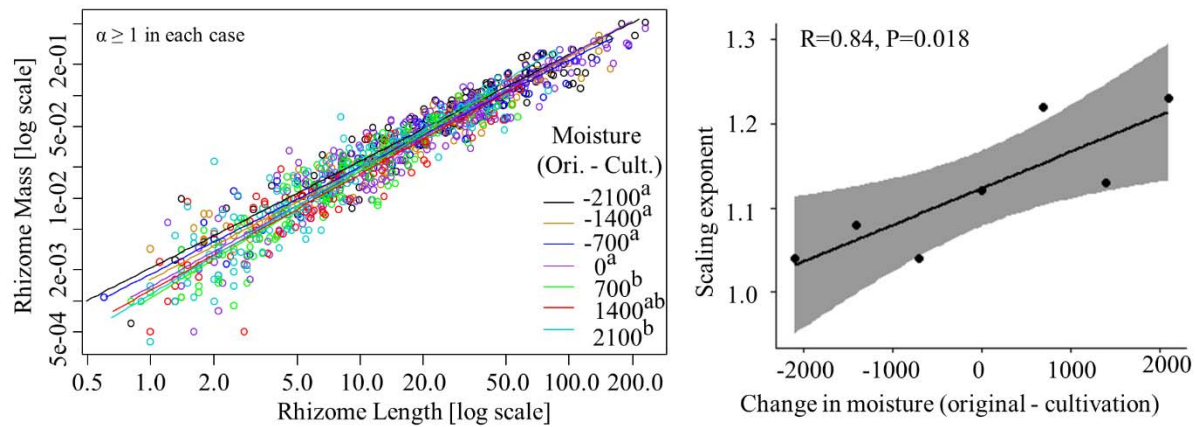
Figure 4: Figure in the left represents scaling relationship between rhizome length (mm) and mass (g) for plants cultivated under changed moisture (mm) conditions (differences in moisture between origin and cultivation climate). The superscript letters represent significant differences in scaling exponent. The right figure represents the change in scaling exponent with changes in moisture (original - cultivation).
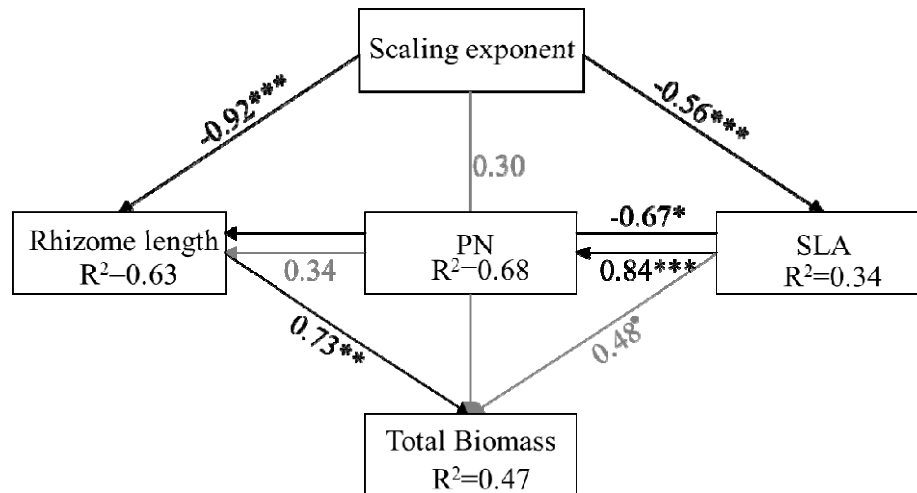
Figure 5: Structural equation model representing the effect of scaling exponent on traits related to plant fitness. PN = net photosynthetic rate, SLA = Specific leaf area (mm^2/mg). Path coefficients between variables are unstandardized regression coefficients and part of the variances explained by the model (R^2) are given under the variable names. The black arrows with regression coefficients in black letters are significant regressions ($p \leq 0.05$), while those in grey are non-significant ($p > 0.05$). *** indicates $p < 0.001$, * indicates $p < 0.05$ and • indicates $p < 0.1$. Goodness of fit statistics of the model are given in supplementary Table S2.5.