

Prediction of wheat grain yield by measuring root electrical capacitance at anthesis**

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Received March 18, 2021; accepted May 12, 2021

Abstract. This methodological study evaluated the efficiency of predicting aboveground biomass and grain yield in field-grown winter wheat by measuring the saturation root electrical capacitance at anthesis. Three cultivars were grown over a three-year period as sole crops and intercropped with winter pea at halved wheat density. The root capacitance readings were converted into saturation root electrical capacitance using the relevant soil water content, according to an empirical function. At plant scale, saturation root electrical capacitance at anthesis showed a significant ($p < 0.001$) linear regression with the total aboveground biomass (R^2 : 0.653-0.765) and grain yield (R^2 : 0.585-0.686) at maturity for each cultivar. At stand scale, both the mean saturation root electrical capacitance and shoot dry mass at anthesis and grain yield varied over the years, and were consistently higher for the intercrops compared to the sole crops. The relative increase in saturation root electrical capacitance due to intercropping corresponded with the changes in shoot dry mass and grain yield, especially in dry years. Saturation root electrical capacitance was significantly correlated with shoot dry mass (R^2 : 0.714-0.899) and grain yield (R^2 : 0.742-0.877) for each cultivar across all cropping systems and years. In conclusion, by mitigating the soil water content effect, the measurement of saturation root electrical capacitance at anthesis is adequate to forecast grain yield and cultivar response to a changing environment.

Keywords: aboveground biomass, intercropping, noninvasive root methods, saturation electrical capacitance, root system size

INTRODUCTION

Larger root system size (RSS) is critical for increased early vigour and water use, it contributes to enhanced grain yield (GY) in crops (Fageria, 2013), thus emphasizing the importance of applying field root phenotyping techniques in breeding programmes (Postic *et al.*, 2019). Nevertheless, as conventional root investigation methods are generally laborious and destructive, and the isolation of the intact root system from field soil is practically impossible, the investigation of roots is often neglected compared to those of shoots.

The measurement of root electrical capacitance (C_R) is a promising, rapid *in situ* technique capable of screening numerous plants at different growth stages. Moreover, the sampled plants can be harvested at maturity to determine GY and can also be used for reproduction (Středa *et al.*, 2020). The C_R method was successfully applied in the field to evaluate the effect of dwarfing genes on the RSS of barley (Chloupek *et al.*, 2006), in order to select barley and wheat genotypes for higher RSS and drought tolerance (Chloupek *et al.*, 2010; Svačina *et al.*, 2014; Heřmanská *et al.*, 2015), to assess the root diversity and water use of wheat varieties (Středa *et al.*, 2012; Nakhforoosh *et al.*, 2014), and to estimate canola RSS in relation to lodging resistance (Wu and Ma, 2016). Some of these studies demonstrated significant relationships between the C_R -based root size and individual GY, particularly in dry environments.

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**This work was funded by the National Research, Development and Innovation Office (NKFIH, grant number OTKA 119475), the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No. 727217 (ReMIX) and a János Bolyai Research Scholarship from the Hungarian Academy of Sciences (2018-2021).

The measurement technique is based on the correlation between RSS variables and the C_R detected between a ground electrode (inserted into the soil) and a plant electrode (fixed on the stem) using a low-frequency alternating current (AC) signal (Chloupek, 1972). Conceptual models consider the roots to be imperfect cylindrical capacitors, in which the amount of electric charge stored by the polarizable membrane dielectrics depends on the root-soil interfacial area (Dalton, 1995). Even though some of the underlying biophysical principles are still unclear and there are uncertainties about the relative contribution of proximal and distal (fine) roots to the magnitude of the C_R detected (Dietrich *et al.*, 2012; Ellis *et al.*, 2013; Cseresnyés *et al.*, 2020; Peruzzo *et al.*, 2020), several pot and field trials have convincingly demonstrated the efficiency of the capacitance method (Středa *et al.*, 2020). One advantage of the technique is that, as the C_R value is affected not only by the size but also by the histological properties of the roots (*e.g.* suberization), the method characterizes both root physiological status and its functionality (Ellis *et al.*, 2013; Cseresnyés *et al.*, 2018). Even though the measured capacitance is very sensitive to soil water content (SWC), this effect can be taken into account by converting the measured C_R to saturation (apparent) capacitance, C_{R^*} , which was detected in experiments on water-saturated soil (Cseresnyés *et al.*, 2018). This adaptation allows us to compare the field data collected at different dates (under different SWC), which was previously considered to be a serious limitation for the capacitance technique (Chloupek *et al.*, 2010; Středa *et al.*, 2012). In this manner, field monitoring revealed that C_R , as a proxy of root activity, peaked during flowering in maize and soybean (Cseresnyés *et al.*, 2018). Minirhizotron and soil core studies verified that wheat root biomass and root length reached a maximum around anthesis, in parallel with the peaks of leaf area, transpiration and water use, and were also significantly correlated with stand GY (Wang *et al.*, 2014; Yang *et al.*, 2018; Postic *et al.*, 2019).

A methodological field study involving three winter wheat cultivars is presented here. As intercropping systems have gained increasing attention in organic farming worldwide due to more efficient, complementary resource use (Bedoussac and Justes, 2011; Lithourgidis *et al.*, 2011), wheat-pea mixtures were tested to compare them with wheat sole crops. Focusing on wheat, RSS was assessed merely on the basis of C_{R^*} measured *in situ* at anthesis. The specific aims of the study were (i) to study the correlation of the individual C_{R^*} values with the total aboveground biomass (TAB) at maturity and also with GY for each wheat cultivar in order to validate the stand-scale results, (ii) to evaluate the effect of pea intercropping with halved wheat density on mean C_{R^*} and the corresponding GY using a stand scale over a three-year period, and (iii) to analyse the relationship between mean C_{R^*} and GY across the cropping systems and years. In brief, the study examined the relevance of the capacitance method in the field, or more precisely, the efficiency with which wheat grain yield may be predicted by measuring the saturation root capacitance (C_{R^*}) at anthesis under different cultivation and climatic conditions.

MATERIALS AND METHODS

The field study was conducted during three winter wheat growing seasons from 2017 to 2020 (referred to as harvest years 2018, 2019 and 2020) in a certified organic field in Martonvásár, Central Hungary (N 47°18', E 18°47', 109 m a.s.l.). The soil was a Haplic Chernozem (36% sand, 41% silt, 23% clay) with a pH value of 7.66, 1.61% CaCO_3 , 3.22% humus, 1887/361/445 mg kg^{-1} total N/P/K and 0.309 $\text{cm}^3 \text{cm}^{-3}$ water content at field capacity. The climate is continental with a mean (1987–2016) annual temperature of 11.0°C (January: -1.0°C , July: 21.2°C) and annual precipitation of 548 mm, of which 193 mm falls during the main crop growing season (March–June; Fig. 1). There were optimal rainfall conditions in 2018. By contrast, late-winter and

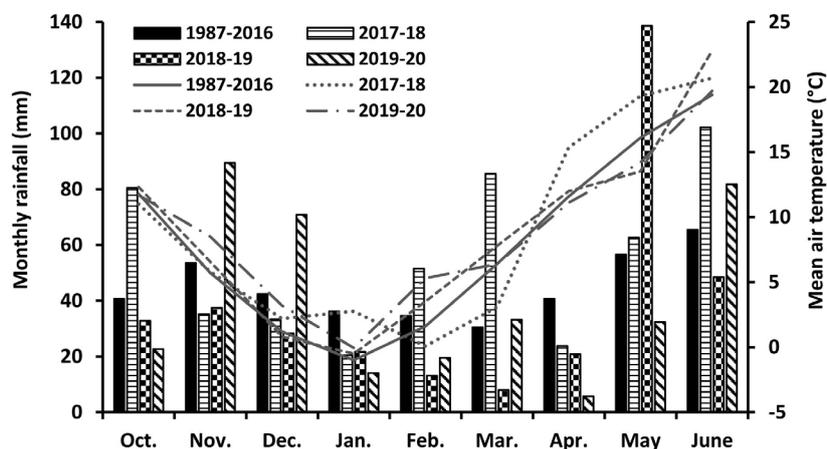


Fig. 1. Monthly rainfall (mm, columns) and mean air temperature ($^\circ\text{C}$, lines) at the experimental site (Martonvásár, Hungary) during the winter wheat growing seasons. The long-term (1987–2016) average is displayed as a reference.

spring droughts occurred in the next two seasons with sufficient precipitation only occurring from early May (flowering stage) in 2019 and from late May (milk stage) in 2020.

Winter wheat (*Triticum aestivum* L.) cultivars ‘Mv Nádor’ (“N”) and ‘Mv Kolompos’ (“K”) and the YQCCP composite population (“C”) were sown in October each year in 6 × 1 m plots with 12 cm row spacing as sole crops (“0”) at a density of 300 seeds m⁻², and at half that density (150 seeds m⁻²) intercrops (“P”) with winter pea (*Pisum sativum* L., cv. Aviron; 50 seeds m⁻²). The three replications of each treatment were randomly arranged in the same field, with each one being surrounded by a 1 m border strip, but in slightly different places each year. Natural fertilizers and artificial chemicals were not used directly, which latter is even banned in organic agriculture.

At the time of anthesis (in early to mid-May, depending on the cultivar and year) 15 wheat plants were randomly selected from the inner rows of each plot. SWC was measured in the 0-12 cm layer 5 cm away from each sample plant (equal to the depth and position of the C_R ground electrode) with a calibrated CS620 portable TDR meter (Campbell Sci. Ltd., Loughborough, UK). The relative water saturation (θ_{rel}) value was calculated by dividing the measured volumetric SWC values (cm³ cm⁻³) by the predetermined saturation water content of 0.476 cm³ cm⁻³ (Cseresnyés *et al.*, 2018). Thereafter, parallel C_R was recorded for the selected plants with a U1733C handheld LCR meter (Agilent Co. Ltd., Penang, Malaysia) at 1 kHz, 1 V AC. The ground electrode was a stainless steel rod 15 cm in length and 6 mm in diameter (303S31; RS Pro GmbH., Gmünd, Austria), pushed vertically into the soil 5 cm from the stem to a depth of 12 cm. The plant electrode was clamped to all of the basal parts of the plant 15 mm above the soil (Svačina *et al.*, 2014) after smearing them with conductivity gel. In order to eliminate the SWC effect, all of the C_R data were converted into C_R^{*}, according to the empirical function: $C_R^* = C_R \cdot 5.807e^{-1.775\theta_{rel}}$, using the relevant θ_{rel} values (for a detailed calculation, see Cseresnyés *et al.*, 2018).

Table 1. Relative soil water saturation (θ_{rel} , mean±SD, n = 45) and root electrical capacitance (C_R, mean±SD, n = 45) measured at anthesis over a three-year period for wheat cultivars ‘Mv Nádor’ (N) and ‘Mv Kolompos’ (K) and the YQCCP composite population (C) grown as a sole crop (N0, K0, C0) or intercropped with pea (NP, KP, CP) in an organic field in Martonvásár, Hungary between 2018-2020. θ_{rel} was the ratio of the detected volumetric soil water content (SWC) to saturation water content (0.476 cm³ cm⁻³)

Treatment	2018		2019		2020	
	θ_{rel}	C _R (nF)	θ_{rel}	C _R (nF)	θ_{rel}	C _R (nF)
N0	0.327±0.019	2.60±0.31	0.730±0.031	5.20±0.73	0.199±0.015	1.99±0.28
NP	0.331±0.022	2.86±0.31	0.739±0.030	6.98±0.54	0.199±0.015	2.90±0.27
K0	0.334±0.019	3.54±0.36	0.640±0.029	5.79±0.73	0.197±0.014	2.46±0.34
KP	0.330±0.016	4.16±0.34	0.627±0.034	6.85±0.72	0.196±0.016	3.57±0.32
C0	0.288±0.028	3.08±0.40	0.837±0.032	8.29±1.19	0.264±0.020	2.69±0.40
CP	0.292±0.027	3.93±0.38	0.834±0.033	9.84±1.11	0.260±0.020	4.09±0.42

After the C_R measurements were complete, five randomly selected wheat plants per plot were cut at ground level, and oven-dried at 70°C until a constant weight was achieved in order to determine shoot dry mass (SDM; ±0.001 g). In the last year (2020) the plants chosen for measuring C_R were individually tagged. At maturity (in early July), the tagged plants were hand harvested and oven-dried to determine TAB, after which they were hand threshed to obtain plant GY. Thereafter, the plots were harvested mechanically, and the wheat grains were separated from the peas and weighed. The mean plant GY was determined for each plot on the basis of wheat seedling density.

The data were analysed with *Statistica* 13.0 software (StatSoft Inc., Tulsa, OK, USA). The unpaired t-test or one-way ANOVA with Tukey’s posthoc test was performed to compare the means of C_R^{*}, SDM and GY (p < 0.05). If the F-test or Bartlett’s test indicated unequal variances, Welch’s t-test or Kruskal-Wallis with Dunn’s posthoc test was used. Linear regression analysis was applied to relate C_R^{*} to TAB, SDM and GY. The resultant regressions were compared using a linear analysis of covariance (ANCOVA).

RESULTS

SWC and thus C_R were found to be highly variable between years and between the dates of anthesis of the cultivars (Table 1). In 2019, a very high SWC was recorded due to heavy rains in May. As the ANOVA showed no significant differences in mean C_R^{*} (n = 15) between the three replicate plots, the data were pooled within each cropping system and year (n = 45) for further analysis.

There were notably significant linear relationships between C_R^{*} measured at anthesis and TAB measured at maturity in 2020 (R² = 0.653-0.765, F: 81.1-140, p < 0.001; Fig. 2a), and between C_R^{*} and GY (R²: 0.585-0.686, F: 60.8-94.0, p < 0.001; Fig. 2b) for all three wheat cultivars. ANCOVA indicated significant differences (p < 0.01) between the cultivars for both the C_R^{*}-TAB and C_R^{*}-GY regressions, with the smallest x-intercept for ‘Mv Nádor’.

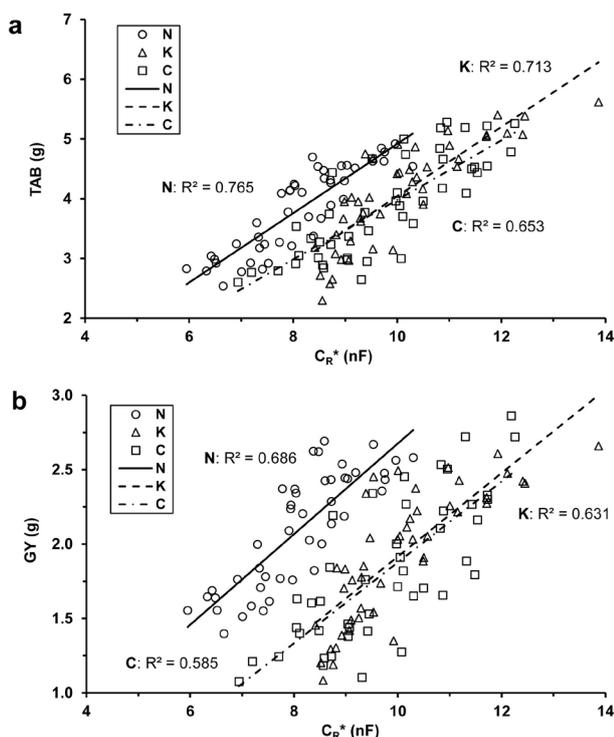


Fig. 2. Relationships between the saturation root electrical capacitance (C_{R^*} , nF) at anthesis, and (a) the total aboveground biomass (TAB, g) at maturity, and (b) grain yield per plant (GY, g) for sole-cropped wheat cultivars ‘Mv Nádor’ (N) and ‘Mv Kolompos’ (K) and the YQCCP composite population (C) grown in an organic field in Martonvásár, Hungary in 2020. Regressions were significant at the $p < 0.001$ level ($n = 45$).

The mean C_{R^*} ranged from 8.10 to 14.94 nF (Fig. 3a). The ANOVA showed a significant year effect in most cases, especially in 2020: C_{R^*} decreased in the sole wheats but increased in the intercrops. According to the t-test, C_{R^*} was significantly higher in mixtures compared to the sole crops for each cultivar and year. Plants harvested at anthesis presented an SDM ranging from 2.33 to 3.72 g (Fig. 3b). The year had an influence on SDM in some treatments, which was reflected in the C_{R^*} results. SDM was consistently higher in the intercrops than in the corresponding sole crops, the only exception being ‘Mv Nádor’ in 2018. The differences were significant for each cultivar in 2019 and even more so in 2020. The mean GY (calculated from the total plot grain mass) ranged from 1.69 to 3.65 g per plant (Fig. 3c). The year had a similar influence on GY as that observed for the C_{R^*} values; however, the effect proved to be insignificant in all cases due to the small number of repetitions ($n = 3$). The GY of intercropped wheat plants was always higher in comparison to their sole-cropped counterparts; the difference was significant in many cases, particularly in 2020.

C_{R^*} , SDM and GY were 9.3–53.0, 7.0–54.6 and 14.9–75.4% higher, respectively, in the half-density mixtures than in the wheat monocultures (Fig. 4a). Again, the exception was ‘Mv Nádor’ which exhibited a 6.0% lower SDM in

the intercrop in 2018. The tendency observed for changes in C_{R^*} was in good accordance with the degree of cultivar response to intercropping in various years, including the reverse ranking of responsiveness in 2018 versus 2019. In 2018, the relative changes in GY due to intercropping were much higher than those in C_{R^*} and SDM. By contrast, C_{R^*} seems to be a more reliable predictor of the alteration in biomass and yield over the next two years. Significant linear relationships were found between C_{R^*} and SDM (R^2 : 0.714–0.899, F : 9.96–35.6, $p < 0.05$, Fig. 4b), and between C_{R^*} and GY (R^2 : 0.742–0.877, F : 11.5–28.4, $p < 0.05$, Fig. 4c) for each wheat cultivar when the data were pooled across cropping systems and years ($n = 6$).

DISCUSSION

The C_{R^*} value proved to be closely correlated with GY per plant in 2020, when the weather was dry until the wheat anthesis stage, with only 55% of the long-term average rainfall from March to May. Previous field studies showed significant relationships between the capacitance-based RSS and GY in dry environments, with an R^2 value of 0.29–0.42 ($p < 0.05$) for wheat (Středa *et al.*, 2012; Heřmanská *et al.*, 2015), and 0.21–0.23 ($p < 0.05$) for barley (Chloupek *et al.*, 2006; Svačina *et al.*, 2014). Moreover, Chloupek *et al.* (2010) reported an R^2 value of 0.63 ($p < 0.05$) for barley in a very dry year. Nevertheless, the correlation was insignificant on occasion (R^2 : 0.11–0.14) under sufficient water conditions, or it was even negative (R : -0.46, $p < 0.05$) for wheat in certain locations in a high-yielding year (Středa *et al.*, 2012).

In the present field study higher R^2 values (0.585–0.686) were obtained for the C_{R^*} –GY regressions, in spite of the rainy weather conditions prevailing during the grain-filling period. This result was most likely due to the use of C_{R^*} , which improved the reliability of measurements by mitigating the effect of variations in SWC. The soil moisture of the plant root zone had a significant positive exponential relationship with the relevant C_R for each wheat cultivar (R^2 : 0.263–0.422, $p < 0.001$, data not shown). The significant cultivar effect on the regression, which may be explained by the relatively short phenotype of ‘Mv Nádor’, indicates that capacitance data may have limited applicability for the comparison of cultivars possessing different morphological properties. As a polygenic trait, GY is controlled by many cofactors beside RSS, however, a greater root system generally leads to improved GY under conditions with limited moisture and/or nutrients (Fang *et al.*, 2017).

The close correlation between C_{R^*} and TAB (R^2 : 0.653–0.765) is partially attributable to the close allometry between root and shoot biomass in monocots such as wheat (Wang *et al.*, 2014), and also, to the size-dependent contribution of the stem portion between the soil surface and the plant electrode to the measured capacitance (Dietrich *et al.*, 2012; Cseresnyés *et al.*, 2020). Chloupek *et al.* (2006) found a weaker relationship between the aboveground

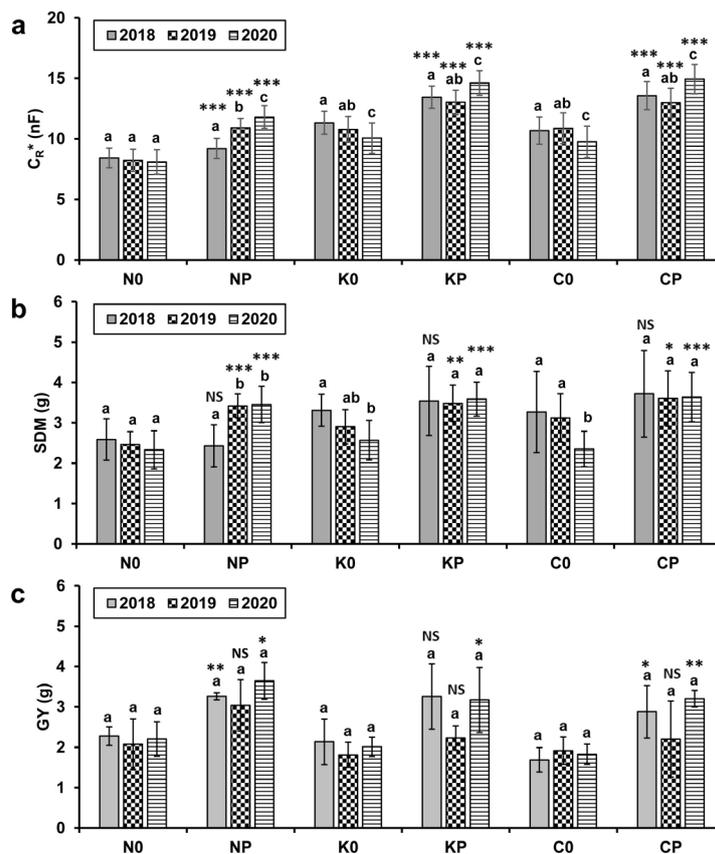


Fig. 3. (a) Saturation root electrical capacitance (C_R^* , mean \pm SD, $n = 45$) at anthesis, (b) shoot dry mass per plant (SDM, mean \pm SD, $n = 15$) at anthesis, and (c) grain yield per plant (GY, mean \pm SD, $n = 3$, calculated from plot grain mass) for wheat cultivars ‘Mv Nádor’ (N) and ‘Mv Kolompos’ (K) and the YQCCP composite population (C) grown as a sole crop (0) or intercropped with pea (P) in an organic field in Martonvásár, Hungary between 2018–2020. Different lower-case letters above the columns indicate significant differences within a cultivar based on treatment combinations ($p < 0.05$) between the years. Asterisks show statistical differences (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS: non-significant) between the cultivar sole crop and the corresponding intercrop in a given year.

biomass and C_R for barley ($R^2 = 0.42$, $p < 0.05$). C_R^* was found to be more closely correlated with TAB than with GY. The reason for this is that the majority of resources are translocated to the grain after the flowering stage, which means that GY is more influenced by post-anthesis conditions (Fageria, 2013), for instance, a sufficient water supply in the present case. The spring drought periods in 2019 and 2020 did not coincide with grain filling, which is a very drought-sensitive stage of wheat growth (Fageria, 2013).

The present experiment generally showed higher C_R^* values as well as higher SDM and GY per plant in intercropped wheat than in the monoculture. In replacement wheat-legume intercrops the cereal is able to compensate for reduced sowing density with increased tillering, grain number and grain weight due to the improved exploitation of growth resources (Bedoussac and Justes, 2011; Monti *et al.*, 2016). The degree of wheat response to half-density intercropping was affected by the cultivar and year of the presented study. The data in the literature suggest that the productivity of cereal-legume mixtures depends on several factors, including their genotypes, cropping design, and relative mixing proportions, as well as cultivation, soil and

climatic conditions (Klimek-Kopyra *et al.*, 2018). A comparison between the three wheat cultivars showed that the relative increase in C_R^* due to intercropping gave a reasonable estimate of the magnitude of changes in SDM and GY in 2019 and 2020. The substantially higher increments in GY compared to those of C_R^* and SDM in 2018 can probably be attributed to the optimal rainfall patterns during the main wheat growing season. Under these conditions, the weaker intraspecific competition arising from the halved sowing density may allow wheat to allocate more assimilates to aboveground parts, including grains, but without a markedly enhanced RSS (Mariotti *et al.*, 2009).

On a stand scale C_R^* values were found to be closely correlated with the SDM ($R^2: 0.714\text{--}0.899$) and GY ($R^2: 0.742\text{--}0.877$) of the cultivars for all cropping designs and years. In a similar manner, Chloupek *et al.* (2010) obtained an R^2 value of 0.60 for the correlation between measured C_R and barley GY in a four-year study. The application of C_R^* therefore seems to have been effective in the present case, particularly in this case considering that the soil was extremely wet at wheat anthesis in 2019, resulting in a much (even 2–3-fold) higher C_R being recorded.

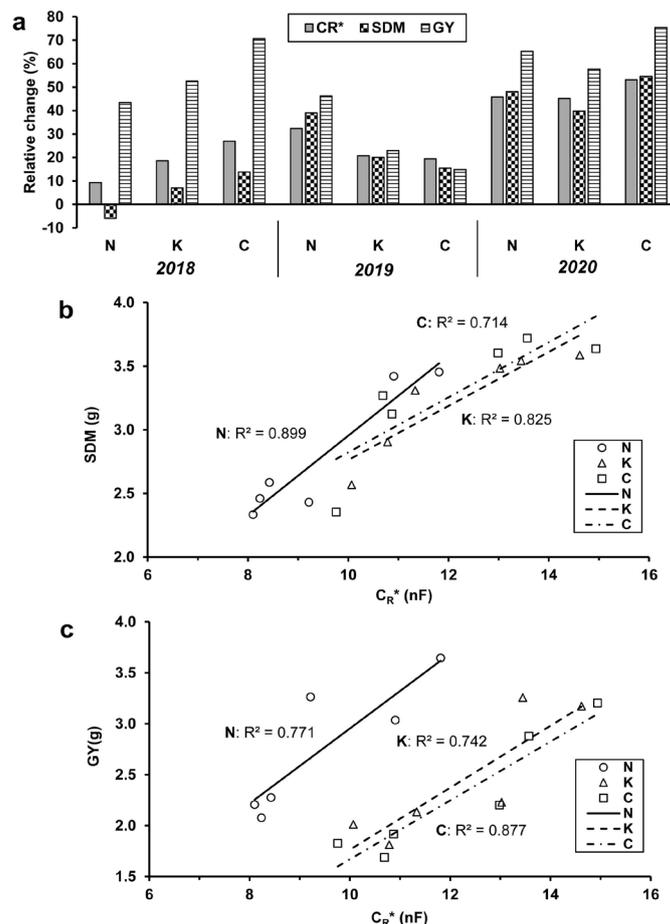


Fig. 4. (a) Relative changes in the saturation root electrical capacitance (C_R^*) at anthesis, shoot dry mass per plant (SDM) at anthesis, and grain yield per plant (GY) as an effect of pea intercropping for wheat cultivars ‘Mv Nádor’ (N) and ‘Mv Kolompos’ (K) and the YQCCP composite population (C) grown in an organic field in Martonvásár, Hungary between 2018–2020. The relationships between C_R^* and (b) SDM or (c) GY for the wheat cultivars are shown above. For each cultivar, data were pooled for the two cropping systems and three years ($n = 6$). Regressions were significant at the $p < 0.05$ level.

CONCLUSIONS

1. This study demonstrated that the saturation root electrical capacitance calculated from root electrical capacitance and the relevant soil water content values measured at wheat anthesis was fairly predictive of individual total aboveground biomass and grain yield data under field conditions.

2. It was found that saturation root electrical capacitance may be used to forecast grain yield in the flowering stage for all of the years studied, even if the soil moisture is variable.

3. The assessment of root size on the basis of saturation root electrical capacitance indicates the different responses of wheat genotypes to altered climatic and cultivation conditions.

4. The *in situ* measurements may provide valuable contributions to crop breeding programmes targeting the selection of cultivars with improved adaptability to changing environments.

Conflict of interest: The authors declare that they have no conflict of interest.

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