



# Article Genetic Divergence and Spatial Configuration Influence the Weed Spectrum, Herbage Yield and Nutritive Quality of Temperate Cowpea

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Abstract: Under a changing climate, the biologically viable management of weeds and the exploration of the genetic divergence of spreading and towering cultivars of forage cowpea in different row configuration systems hold the potential to boost sustainable feed supply for dairy animals. A field study was undertaken to sort out the most nutritive and high-biomass-producing cultivar (Cowpea-,2007 and Rawan-,2010) of cowpea and optimize the row configuration ( $R \times R$  of 15, 30, 45 and 60 cm) to manage the weed spectrum. The results revealed that Rawan-2010 remained superior in the 15 cm row configuration by recording 39% lesser weed density (WD) than the corresponding value recorded by the same cultivar sown in the 60 cm row configuration. The same treatment combination recorded a 20% lesser fresh weed weight than Cowpea-,2007 sown in the same row configuration, while it exhibited a 5.6 g m<sup>-2</sup> lesser corresponding value of dry weed weight. In contrast, Cowpea-2010 sown in the 45 cm row configuration recorded the maximum yield attributes (stem girth, leaf and branch numbers, leaf area, fresh and dry weights per plant), except plant height (PH), which resulted in 7% and 13% higher green herbage yield (GH) and dry matter biomass (DM), respectively, than the same cultivar sown in the 30 cm row configuration. Pertaining to nutritional value, Rawan-2010 in the 45 cm row configuration yielded the maximum crude protein and minimum crude fiber content, while the same cultivar gave the greatest ash content in the wider row spacing. With GH, the correlation analyses indicated an antagonistic association for PH, a moderately linear relationship between stem girth and branch numbers and a strong direct association between leaf area and fresh plant weight.

Keywords: planting geometry; biomass; crude protein; correlation analysis; leguminous forages

## 1. Introduction

Globally, the skyrocketing population necessitates the proportional enhancement of milk and meat production, which require a sustainable supply of quality forages in abundant quantities throughout the year [1]. Among forage crops, cereals such as sorghum, maize, oat, barley, and millets yield copious quantities of green herbage; however, these cereals have low protein and digestibility. Thus, in order to maintain milk production, dairy animals need to be fed expensive protein additives that lead to a significant hike in the cost of production and a depletion of net returns [2]. Legumes hold bright perspectives to overcome the nutritional quality concerns related to forages due to having superior nutritional quality along with the potential to gain nitrogen (N) through the biological



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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). N fixation process. Among legume forages, cowpea (*Vigna unguiculata* (L.) Walp) is of pivotal pertinence in the USA, many African countries, China and especially in South Asian countries (Pakistan, India and Bangladesh) [3]. It encompasses numerous advantageous characteristics, including unmatched drought tolerance, superior adaptability to harsh climatic conditions, and the unique option to be grown as a dual-purpose crop (forage and grain). Additionally, cowpea has the potential to be adjusted in a variety of farming systems (irrigated or rainfed, arid or semi-arid, tropical or temperate) owing to its established tolerance against abiotic stresses such as drought, heat, salinity, soil erosion and, more importantly, its unmatched ability to thrive well in toxic soils [4–7].

Nevertheless, in order to become a viable alternative to traditional multi-cut leguminous forages (clover species, alfalfa, etc.), cowpea cultivation must demonstrate a competitive yield advantage in terms of higher herbage yield and nutritive quality. In addition, the changing climate, which has been characterized by global warming and the shifting of rainfall patterns and seasonal distributions, necessitates the cultivation of drought- and heat-resilient crops like cowpea in rainfed farming systems of temperate regions [8–10]. However, cowpea forage yield in temperate areas has remained suboptimal compared to other single-cut forages, primarily owing to low-yielding cultivars and outdated agronomic production technology packages. Presently, towering and spreading types of cowpea cultivars are available in Pakistan, having moderate biomass yield potential in the range of 14–30 tons per hectare with an appropriate level of nutritional quality [11,12]. However, to the best of our information, research findings on the comparative performance of forage cowpea cultivars in terms of herbage yield and nutritional quality under temperate conditions are very scant. Besides its genetic potential, agronomic production technology, especially the row configuration of towering and spreading types of cultivars, imparts significant influence on weed composition, crop plants' growth and herbage yield [13–16]. Row configuration alterations have been effectively used to manage soil moisture deficiency through skipping one or multiple rows of the crop in what is commonly referred to as a skip row configuration, while on the other hand, adding one or more rows by reducing inter-row spacing is called an additive row configuration. There is a serious lack of fieldtrial-based evidence regarding row configuration optimization for boosting herbage yield and nutritional quality traits of cowpea cultivars.

As a result of the changing climate, infestations of indigenous and exotic weeds have become robust in temperate regions, necessitating the evaluation of biologically viable ways to keep them below a threshold level. Previous studies demonstrated that altering row configurations, especially closer ones, offered weed suppression in a biologically viable way and also resulted in the robust growth of plants on either side of the rows [17,18]. Likewise, wider row spacing for grain crops recorded 21–43% higher weed density owing to the agro-botanical superiority of weeds in acquiring growth resources like moisture, nutrients and solar radiation [19]. Additionally, it was revealed that cowpea-sorghum intercropping in 30 cm spatial arrangements resulted in significantly lesser (57%) density and biomass production (29% lesser fresh weight and 37% lower dry weight compared to wider row configurations) of weeds [19–21]. In addition to weed management, narrow row configurations remained effective in controlling wind erosion, aided in conserving moisture and reduced crusting of the soil surface, which led to improved soil health and structure. Additionally, a narrow planting configuration served as a viable way to use the available moisture efficiently in dry farming [22,23]. Moreover, it was also observed that changes in plant configuration imparted a significant influence on leaf area index and canopy development through the alteration of evapotranspiration partitioning between the soil surface and crop plants. These findings were supported by another study, whereby Staggenborg et al. [24] attained higher biomass yields from narrow rows in comparison to wider rows under optimum fertility and moisture conditions. It was also inferred that under moisture-deficient conditions, no significant differences in biomass yield by different row configuration treatments was evident. In contrast, Bandaru et al. [25] found superior herbage and grain yields from narrow rows of crops coupled with wide intra-row spacing

among seedlings. However, it was suggested that the clumping of narrow and wide row configuration remained superior only under sub-optimal conditions, while this effect lost superiority as growth conditions were optimized. M'Khaitir and Vanderlip [26] reported that increasing the plant population remained effective in boosting plant growth and biomass production, especially when soil moisture remained sufficient. Again, conclusive findings are lacking pertaining to row configuration optimization and its impact on weed density, herbage yield and the nutritional quality of cowpea in temperate regions.

In light of the changing climatic scenario and emerging market opportunities in the dairy industry, we set out to reinvestigate the potential fit of forage cowpea in the temperate Himalayan region of Pakistan. However, the prime need of the time is to bridge research and knowledge gaps pertaining to the interactive effect of genetic potential and row configuration on weed density, growth attributes, biomass yield and the nutritional quality of cowpea. To achieve this goal, we hypothesized that harmonizing row configuration with genetic divergence (towering- or spreading-type growth habit) might boost vegetative growth, herbage yield, quality attributes and weed suppression through the effective use of farm inputs and environmental resources in spatio-temporal dimensions. In contrast, temperate climatic conditions could potentially restrict the expression of genetic potential and neutralize the influence of row arrangement on the growth, yield and quality traits of cowpea. Thus, this field trial was undertaken with the objective of harmonizing the row configuration of cowpea cultivars having varying growth habits in rainfed farming under a temperate climate.

#### 2. Materials and Methods

#### 2.1. Meteorological Features and Physico-Chemical Description of Experimental Locality

The field trial was executed at the research area (main campus) of the University of Poonch Rawalakot, Azad Jammu and Kashmir, Pakistan, during 2018–2019. The geographical coordinates [27] of the study site are presented in Figure 1. The test crop (cowpea) was sown after the harvesting of winter wheat on June 22 and 25 of 2018 and 2019, respectively. The meteorological features regarding temperature and rainfall of the study site during the crop growth season (mean values of both years) are presented in Figure 2. The study locality entails rainfed farming systems receiving sufficient precipitation during the crop growth cycle to support the economical production of crops like maize, soybean, sorghum, and a variety of vegetables that are primarily grown at the subsistence level.

Prior to the cultivation of the test crop (cowpea), the physicochemical analyses of the experimental block were performed by collecting soil samples from two depths (0–15 cm and 15–30 cm) from four corners and the middle of the experimental block. Subsequently, the soil samples (belonging to both soil depths) were homogenized thoroughly by hand mixing, and thereafter, the samples were shade dried, grounded and sieved (using a sieve having a 2 mm pore size). For the estimation of pH, the soil was mixed with water (1:2.5 ratios) to prepare the paste that was subjected to the glass electrode for determining the pH [28]. The electrical conductivity (EC) of the soil samples was estimated using a conductivity meter [29-33]. In addition, organic carbon (OC) content was evaluated using the wet oxidation method, while the Walkley-Black protocol was followed for assessing the organic matter (OM) content [29]. Moreover, total nitrogen (N) was estimated with the help of the Kjeldahl apparatus for distillation and  $H_2SO_4$  (concentrated acid) titration [30]. Phosphorous (P) was determined by using Olsen's method, which entails the reaction of 0.5 N NaHNO<sub>3</sub> at 8.5 pH with a soil:extractant paste (1:10 ratio) and the subsequent use of spectrophotometer (882 nm) in a system containing  $H_2SO_4$  [31]. Finally, potassium (K) availability was estimated by an ammonium acetate extraction (shaking soil samples with an ammonium acetate solution of 0.5 M for 30 min) method that caused K<sup>+</sup> ion displacement, and a flame photometer was used for their detection. For recording micronutrient concentrations in soil samples, an extraction method encompassing ammonium acetate solution ( $CH_3COONH_4$ ) was reacted with soil paste (pH = 3.0) for iron (Fe) estimation. Thereafter, a colorimetric method along with a spectrophotometer (510 nm

wavelength) was put into practice to determine Fe content. Moreover, the concentration of micronutrients, including boron (B), zinc (Zn), copper (Cu) and manganese (Mn), were estimated by following the extraction method that involved diethyle-netriaminepentaacetic acid [32–34]. The experimental soil's texture was loam, having a pH and OM of 7.8 and 1.05%, respectively, indicating the dire need to appropriately fertilize the soil to achieve the potential yield. The bulk density of the experimental soil was 1.24 cm<sup>-3</sup>, while the EC was 0.45 dS m<sup>-1</sup>, indicating the soil was normal without salinity. As far as the macronutrients of the soil samples were concerned, the NPK concentrations remained at 87, 5.8 and 183 mg kg<sup>-1</sup>, respectively. The micronutrients were in an appropriate range, such as B (1.18 mg kg<sup>-1</sup>), Mn (19.2 mg kg<sup>-1</sup>), Fe (14.2 mg kg<sup>-1</sup>), Cu (1.82 mg kg<sup>-1</sup>) and Zn (1.29 mg kg<sup>-1</sup>).



**Figure 1.** The location of the trial map (Rawalakot, District Poonch, Poonch, Azad Jammu and Kashmir, Pakistan) prepared for this study with the help of QGIS software (version 3.24.3, Bern, Switzerland), whereby the red star indicates the approximate location of the trial and the half-arrow depicts the North direction.



**Figure 2.** The study area's (Rawalakot, District Poonch, Azad Jammu and Kashmir, Pakistan) meteorological features (temperature and rainfall) during the course of field trials.

#### 2.2. Details of Treatments and Experiment's Execution

The field experiment was constituted of cowpea cultivars (Cowpea-,2007 and Rawan-,2013) of varying growth habits (spreading and towering ones) and different row configurations including  $R \times R = 15$  cm,  $R \times R = 30$  cm,  $R \times R = 45$  cm and  $R \times R = 60$  cm. In this way, there were eight treatment combinations in total. The execution of the field trial was performed as per randomized complete block design (RCBD) in a regular arrangement. The replications of all experimental units were maintained in triplicate. The experimental units had a net plot size of  $3.6 \text{ m} \times 5 \text{ m}$ , maintained after excluding the land area occupied by walking paths of 0.60 m width and 0.45 m wide bunds surrounding the experimental plots. In addition, fellow areas of 0.60 m were maintained among the experimental units, while a 5 m fellow area was kept among replications. Regarding fertilization, the co-application of organic (chicken manure at the rate of 5 tons  $ha^{-1}$ ) and mineral fertilizer (DAP at the rate of 60 kg  $ha^{-1}$ ) was done as a basal dose owing to rainfed conditions. The seeds of cowpea cultivars were hydro-primed (by pre-soaking seeds dipping in sterilized water for 12 h) in order to achieve rapid and vigorous seed germination as recommended by Iqbal et al. [35]. Thereafter, seeds were shade dried on clean muslin cloth sheets and subsequently stored at 10 °C.

Regarding seed-bed preparation, a tractor-driven common cultivator was used to plough the field thrice, while planking (wooden plank) followed each ploughing. A fine seedbed was prepared, having been thoroughly pulverized. Cowpea cultivars were sown as per treatment using a 30 kg seed rate ha<sup>-1</sup> in the last week of June during both years using a single-row hand drill, and plant-to-plant spacing was maintained at 15 cm.

## 2.3. Response Variables Recordings

For recording the data of the response variables, ten plants were randomly selected from the central rows of experimental units, and their averages were then computed for further analyses. Plant height was determined with the help of a tailor's tap from the plant base to the tip of the uppermost leaf, and leaf area was estimated using a portable digital leaf area meter. The stem girth of cowpea plants was recorded using a vernier caliper. The green herbage yield was estimated after harvesting all plants in every unit that were separately bundled and weighed using a spring balance in the field. Thereafter, the biomass yields of experimental units were converted into a hectare basis by following Equation (1). For the estimation of crude protein content, a macro-KJeldahl apparatus was used for nitrogen measurement, which was multiplied by a constant of 6.25. Likewise, the  $H_2SO_4$  and NaOH digestion method was followed for assessing crude fiber contents. In addition, the soxhlet extraction methodology was followed in order to determine the extractable ether percentage of forage samples of all treatments preserved in triplicate. Finally, ashing (at 600 °C) of forage samples was performed as per the muffle furnace technique for the calculation of total ash contents [35,36].

Herbage yield of cowpea = Yield per plot 
$$\times$$
 10,000 m<sup>2</sup>  
Plot area (m<sup>2</sup>) (1)

#### 2.4. Statistical Analyses

The data were recorded for all response variables under study. These data were thoroughly arranged and subsequently subjected to statistical analyses using Bartlett's test, which exhibited a non-significant impact of the year, and thus, yearly data transformation into the mean values was performed for sorting out the statistical significance among employed treatments. After that, Fisher's analysis of variance (ANOVA) was put into use for estimating overall significance, while a comparison for treatment means was made using Tukey's honest significant difference (HSD) test at the level of probability of 5% using the SAS statistical package (9.2 Version, SAS Institute, Cary, NC, USA) [37,38].

## 3. Results and Discussion

#### 3.1. Weeds Density and Biomass

During the course of field investigation, different types of weeds were identified in experimental units, including Conyza bonariensis, Parthenium hysterophorus, C. canadensis, Cannabis sativa, Tagetes minuta, C. japonica, Xanthium strumarium, Centaurea cyanus, Lamium album, Leonurus cardiac and Strobilanthes urticifolia. The findings revealed that weed density (WD) and biomass varied significantly among experimental units encompassing treatment combinations of different cowpea cultivars and row configurations (Figure 3). It was observed that Cowpea-,2007 in all row configurations recorded comparatively higher WD, especially in the row configuration of 60 cm ( $C_1P_4$ ), while narrow row spacing (15 cm) ( $C_1P_1$ ) allowed much lesser (39%) WD (Figure 3A). In a similar fashion, the row configuration of 45 cm  $(C_1P_3)$  recorded comparatively greater WD than 30 cm, while it exhibited a significantly lower corresponding value in comparison to the row configuration of 60 cm. In contrast, cowpea cultivar Rawan-2010 sown in the 15 cm row configuration ( $C_2P_1$ ) outperformed Cowpea-2007 by recording a more significantly meager WD than the same cultivar sown in the 60 cm row spacing ( $C_2P_4$ ). Following the trend, this cultivar also recorded a higher number of weeds in a wider row configuration of 60 cm compared to the 45 cm row configuration ( $C_2P_3$ ). Pertaining to the fresh weight of weeds (WFW), cowpea cultivar Cowpea-,2007 sown in the row configuration of 60 cm resulted in the maximum values of weed fresh weight, while the same cultivar recorded lesser WFW in the 30 cm row configuration (Figure 3B). However, Rawan–,2010 remained superior by exhibiting a comparatively lesser WFW, especially in the row configuration of 30 cm  $(C_2P_1)$ , which was 20% lesser than Cowpea-,2007 sown in the same row configuration. In addition, Rawan–,2010 sown in the 45 cm row configuration outperformed the 60 cm row spacing, but it remained inferior to the 30 cm spacing as far as WFW in forage cowpea was concerned (Figure 3C). Moreover,  $C_1P_4$  (Cowpea-,2007 sown in the 60 cm row configuration) recorded the maximum weed dry weight (WDW), which was 7.8 g m<sup>-2</sup> greater than the WDW produced by the same cultivar in the 30 cm row configuration. However, Rawan-2010 remained superior in the 15 cm row configuration  $(C_2P_1)$  by recording the minimum WDW, which was 5.6 g m<sup>-2</sup> lesser than the same cultivar sown in a wider row spacing of 60 cm. Overall, Rawan-,2010 in the 30 cm row configuration recorded a 17% less WDW in comparison to Cowpea-,2007 sown in the corresponding row spacing. These findings corroborate with those of Abbas et al. [39], who opined that wider row spacing significantly enhanced the weed density owing to the greater space available for weed seeds to germinate and thrive vigorously, which led to greater intra-species competition

and, ultimately, cowpea plants suffered adversely. It was also inferred that although closer row spacing reduced weed density, it also resulted in higher plant-plant competition for growth resources, which led to stunted plant height and lower vegetative growth traits of crop plants. Similarly, in our trial, the spreading type of cultivar in a closer row spacing of 30 cm provided lesser space for weed growth, which reduced weed density. Additionally, intense competition for limited growth resources, especially moisture and nutrients in closer spaced row configurations, might also be attributed to lower weed fresh weight [14–17]. In agreement with our findings, it was concluded that sub-optimal wider planting arrangements (60 cm and higher) resulted in significantly higher weed density (23–29%) and dry weight (34–41%) owing to superior agro-botanical traits of weeds, which promoted the vigorous growth of weeds by virtue of their higher nutrients and moisture uptake compared to crop plants [15,21].



Figure 3. Cont.

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**Figure 3.** Weed infestation as indicated by (**A**) the density of weeds, (**B**) fresh weight of weeds and (**C**) dry weight as influenced by the genetic divergence of cowpea cultivars and row configuration under a temperate climate. C<sub>1</sub> = cowpea cultivar Cowpea–2007, C<sub>2</sub> = cowpea cultivar Rawan–2010, P<sub>1</sub> = R × R of 15 cm, P<sub>2</sub> = R × R of 30 cm, P<sub>3</sub> = R × R of 45 cm, P<sub>4</sub> = R × R of 60 cm. Column bars having different letters significantly vary at p = 0.05.

## 3.2. Yield Attributes

The research findings showed the significant influence of cowpea cultivars' genetic divergence and row configuration on the vegetative growth traits of cowpea plants under temperate climatic conditions (Table 1). As far as plant height (PH) was concerned, Cowpea-,2007 recorded the tallest plants, especially in the row configuration of 30 cm  $(C_1P_2)$ , and it was followed by the same cultivar sown in the 45 cm row spacing, which recorded a 4% lesser PH than  $C_1P_2$ . The Rawan-2010 exhibited a significantly lesser PH, as  $C_2P_3$  gave 40% less PH compared to  $C_1P_2$ ; however, it remained superior to  $C_1P_4$ . In contrast to PH, Rawan-,2010 outmatched Cowpea-,2007 sown in all row configurations in terms of stem girth (Sg). In particular, the 45 cm row configuration recorded the maximum Sg. It was followed by the same cultivar sown in the 30 cm row spacing, while Cowpea-,2007 exhibited the most thin stemmed plants, particularly in the row configuration of 60 cm. Pertaining to the number of branches (BN) and leaves (LN) per plant of cowpea, Rawan -,2010 in the 45 cm row configuration ( $C_2P_3$ ) recorded the maximum values, which were 8% and 6%, respectively, higher than the following treatment combination of  $C_2P_2$ . The minimum values of BN and LN were exhibited by  $C_1P_1$ , which were 79% and 87% lower than the best performing treatment combination of  $C_2P_3$ . Regarding the leaf area (La) per plant at 56 DAS (La<sub>1</sub>) and 75 DAS (La<sub>2</sub>), the maximum values of La<sub>1</sub> and La<sub>2</sub> were exhibited by the cowpea cultivar Rawan-2010 planted in the row configuration of  $R \times R$  of 45 cm (C<sub>2</sub>P<sub>3</sub>), while the following treatment combination of  $C_2P_2$  produced 84% and 79% lesser values of La<sub>1</sub> and La<sub>2</sub> in comparison to  $C_2P_3$ . The minimum La at both recordings was demonstrated by Cowpea –,2007 sown in a narrow row configuration of 15 cm ( $C_1P_1$ ) by recording a 53% and 51% fewer La<sub>1</sub> and La<sub>2</sub> than the most well-performing treatment combination of  $C_2P_3$ . Interestingly, the narrowest row configuration of Cowpea-2007 remained statistically at par with the wider row spacing  $(C_1P_4)$  as far as La<sub>1</sub> and La<sub>2</sub> were concerned (Table 1). The research findings of this field trial corroborate with those of previously reported conclusions [40-42], whereby plant height was reported to be a genetically controlled trait, and appropriate agronomic management, especially optimal planting density, also remained significantly effective in producing taller

plants. However, in our study, Cowpea-2007 remained taller primarily owing to its genetic traits compared to the spreading type of Rawan-2007, as both cultivars were subjected to similar row configurations in the field. Besides genetics, the 45 cm row configuration might have resulted in the better attainment of growth resources like solar radiation, macro- and micro-nutrients, and moisture, which promoted the stem diameter and number of branches per plant [43–47]. Moreover, the significantly lower leaf area in wider row configurations might be attributed to higher weed densities, which could have restricted nutrient supply to crop plants, and ultimately, a reduced leaf area was recorded for both cultivars of forage cowpea [14,15,48].

**Table 1.** Yield traits of cowpea under the interactive effect of genetic divergence and row configuration in a temperate climate.

Treatments	Plant Height (cm)	Stem Girth (cm)	Branches Number per Plant	Leaf Number per Plant	Leaf Area per Plant (cm <sup>2</sup> ) at 56 DAS	Leaf Area per Plant (cm <sup>2</sup> ) at 70 DAS
$C_1P_1$	$68.3\pm0.28~^{d}$	$5.16\pm0.13~^{e}$	$9.3\pm0.04~^{g}$	$24.3\pm0.09~^{\rm f}$	$75.9\pm0.24^{\text{ g}}$	$91.1\pm0.51$ $^{\rm h}$
$C_1P_2$	$77.7\pm0.19$ $^{\rm a}$	$6.01\pm0.55~^{\rm d}$	$12.6\pm0.51$ $^{\rm d}$	$27.6\pm0.50~^{\rm d}$	$88.6\pm0.39~^{\rm e}$	$109.4\pm0.32$ $^{\rm e}$
C <sub>1</sub> P <sub>3</sub>	$74.8\pm0.81~^{b}$	$5.13\pm0.92~^{\rm e}$	$11.1\pm0.63~^{\rm e}$	$26.3\pm0.15~^{\rm e}$	$79.3\pm0.63~^{\rm f}$	$100.9\pm0.18~^{\rm f}$
$C_1P_4$	$58.3\pm0.64~^{\rm c}$	$4.66\pm0.28~^{f}$	$10.7\pm0.45~^{\rm f}$	$24.3\pm0.45~^{\rm f}$	$76.3\pm0.45^{\text{ g}}$	$94.7\pm0.29~^{\rm g}$
$C_2P_1$	$53.3\pm0.37~^{g}$	$6.03\pm0.35~^{d}$	$14.0\pm0.07~^{\rm c}$	$40.3\pm0.27$ $^{\rm c}$	$93.3\pm0.17~^{d}$	$115.0\pm0.11~^{\rm d}$
C <sub>2</sub> P <sub>2</sub>	$54.7\pm0.19~^{\rm f}$	$6.65\pm0.41~^{\rm b}$	$15.5\pm1.01~^{\rm b}$	$44.6\pm0.11~^{\rm b}$	$107.6\pm0.53$ $^{\rm b}$	$126.6\pm0.65~^{b}$
C <sub>2</sub> P <sub>3</sub>	$55.8\pm0.41~^{\rm e}$	$7.07\pm0.22~^{a}$	$16.7\pm0.50$ $^{\rm a}$	$46.1\pm0.50~^{\rm a}$	116.7 $\pm$ 0.21 $^{\rm a}$	137.1 $\pm$ 0.29 $^{\rm a}$
$C_2P_4$	$53.1\pm0.34~^{\rm g}$	$6.10\pm0.19$ c $^{\rm c}$	$15.3\pm0.83^{\text{ b}}$	$41.3\pm0.33~^{\rm c}$	$100.3\pm0.32~^{\rm c}$	$121.5\pm0.32~^{\rm c}$

 $C_1$  = cowpea cultivar Cowpea-2007,  $C_2$  = cowpea cultivar Rawan-2010,  $P_1$  = R × R of 15 cm,  $P_2$  = R × R of 30 cm,  $P_3$  = R × R of 45 cm,  $P_4$  = R × R of 60 cm, DAS = days after sowing. Values having different letters within same column vary significantly at p = 0.05.

#### 3.3. Plant Fresh and Dry Weights, Green Herbage Yield and Dry Matter Biomass

The results of this field trial demonstrated that genetic divergence and row configuration significantly influenced fresh plant weight at 60 DAS ( $WF_1$ ) and 80 DAS ( $WF_2$ ), dry weight at 60 DAS (WD<sub>1</sub>) and 80 DAS (WD<sub>2</sub>), green herbage (GH) and dry matter (DM) yields (Table 2). As far as  $WF_1$  and  $WF_2$  were concerned, Rawan-2010 sown in 45 cm row spacing  $(C_2P_3)$  remained superior to the rest of the row configurations and Cowpea-2007 under all row spacings. This treatment combination was followed by C<sub>2</sub>P<sub>2</sub>; however, it produced 6% and 5% lesser WF<sub>1</sub> and WF<sub>2</sub>, respectively, compared to  $C_2P_3$ . The corresponding minimum values of WF1 and WF2 were recorded for Cowpea-2007 sown in the closest row configuration of  $R \times R$  of 15 cm (C<sub>1</sub>P<sub>1</sub>), which were 24% and 22%, respectively, less than  $C_2P_3$ . Regarding WD, the maximum WF<sub>1</sub> and WF<sub>2</sub> were exhibited by the cowpea cultivar of Rawan-2010 sown in the row configuration of 45 cm, while the lowest corresponding values were given by Cowpea–2007 planted in 15 cm R  $\times$  R. In a similar fashion, Rawan-2010 remained outmatched, especially in the 45 cm row configuration ( $C_2P_3$ ) by recording the maximum GH and DM, while it was followed by  $C_2P_2$ , which produced 7% and 13% lesser GH and DM, respectively. Cowpea-,2007 sown in 15 cm row spacing could not perform on par with the rest of the treatments and recorded the minimum GH and DM, which were 35% and 68% lesser than  $C_2P_3$ . These findings are in contrast to Bange et al. [49], who reported no significant influence of skip or additive row configuration on plant growth and weights; rather, it was opined that row spacing was usually governed by farming needs like machinery use considerations. However, in our field trial, row configuration influenced solar radiation interception owing to the towering and spreading nature of the cultivars, which promoted plant fresh and dry weights in 45 cm spacing, while too close and more wide row configurations reduced plant weights owing to the intense competition for growth resources and weed interference, respectively, which led to significantly lower

herbage yield and dry matter production. These findings support previously reported results [50–54] whereby changing the row spacing modified the water reserves available in the soil along with the pattern by which moisture became available and was uptaken by the crop plants. It was also suggested that narrow row spacing could boost plant growth in soils having poor soil structure that restrict root exploration into the skip area, and thus, narrower row spacing could record higher biomass yield owing to the superior plant population [55–57]. Conversely, narrower row spacing might also be practiced on goodstructured soils having higher moisture availability because it might compensate for closer row spacing, and ultimately, plants with higher fresh and dry weights could be produced, as reported by Kerby et al. [58]. However, it was demonstrated that plant population impact in varying row configurations (replacement and additive) showed no consistent association between plant growth and plant population [56,59]; however, we may assume that these insignificant results were owing to the testing of towering type cultivars, as otherwise, an amalgamation of spreading- and towering-type cultivars could have responded positively to row configurations in terms of plant fresh and dry weights along with green herbage production [54,57,60]. Moreover, better yield attributes by Rawan-2010 in the row configuration of 45 cm might be attributed to the significantly higher green herbage yield and dry matter production, especially regarding higher plant fresh and dry weights. The greater genetic potential, higher capacity to intercept photosynthetically active radiation, improved root architecture for the uptake of moisture and nutrients along with the greater leaf area that triggered biosynthesis of carbohydrates were reported to be prime factors in boosting the biomass production of forage legumes like cowpea, soybean, cluster bean and ricebean [47,50,54,61–65].

**Table 2.** Fresh and dry weight per plant, green herbage yield and dry matter yield under the interactive effect of genetic divergence and row configuration in a temperate climate.

Treatments	Plant Fresh Weight (g) at 60 DAS	Plant Fresh Weight (g) at 80 DAS	Plant Dry Weight (g) at 60 DAS	Plant Dry Weight (g) at 80 DAS	Green Herbage Yield (t ha <sup>-1</sup> )	Dry Matter Yield (t ha <sup>-1</sup> )
$C_1P_1$	$86.2\pm0.35~^{\rm f}$	$100.6\pm0.61~^{\rm f}$	$28.1\pm0.11~^{\rm f}$	$34.0\pm0.28~^{e}$	$12.3\pm0.24$ $^{\rm f}$	2.9. $\pm$ 0.23 <sup>g</sup>
$C_1P_2$	$92.1\pm0.17~^{d}$	$104.2\pm0.28~^{d}$	$30.9\pm0.42~^{d}$	$37.7\pm0.07~^{d}$	$15.2\pm0.51$ $^{\rm c}$	$3.4\pm0.50~^{\rm e}$
C <sub>1</sub> P <sub>3</sub>	$88.6\pm0.63~^{\rm e}$	$102.9\pm0.19$ $^{\rm e}$	$29.8\pm0.29~^{\rm e}$	$37.2\pm0.23~^{\rm d}$	$14.1\pm0.16~^{\rm d}$	$3.1\pm0.83~^{\rm f}$
$C_1P_4$	$88.1\pm0.55~^{ef}$	102.1 $\pm$ 0.26 $^{\rm e}$	$29.1\pm0.09~^{\rm e}$	$35.1\pm0.41~^{\rm de}$	$13.0\pm0.45$ $^{\rm e}$	$3.0\pm0.45~^{d}$
$C_2P_1$	$101.2\pm0.13$ $^{\rm c}$	$116.9\pm0.41~^{\rm bc}$	$34.7\pm0.47$ $^{\rm c}$	$41.9\pm0.38~^{cd}$	$14.3\pm0.37~^{\rm d}$	$3.9\pm0.37~^{c}$
$C_2P_2$	$102.1\pm0.42$ $^{\rm b}$	$117.9\pm0.55~^{\rm b}$	$35.1\pm0.461~^{b}$	$43.1\pm0.23~^{\rm b}$	$15.6\pm0.11$ $^{\rm b}$	$4.4\pm0.16$ $^{\rm b}$
C <sub>2</sub> P <sub>3</sub>	$107.9\pm0.69$ $^{\rm a}$	$123.8\pm0.12$ $^{\rm a}$	$38.6\pm0.25$ $^{\rm a}$	$47.9\pm0.30~^{\rm a}$	$16.7\pm0.50$ $^{\rm a}$	$4.9\pm0.51~^{\rm a}$
$C_2P_4$	$101.4\pm0.29\ensuremath{^{\rm c}}$ c	$114.0\pm0.19$ c	$34.6\pm0.33~^{\rm c}$	$42.0\pm0.14~^{\rm c}$	$15.3\pm0.63$ $^{\rm c}$	$4.0\pm0.63$ c

 $C_1$  = cowpea cultivar Cowpea–2007,  $C_2$  = cowpea cultivar Rawan–2010,  $P_1$  = R × R of 15 cm,  $P_2$  = R × R of 30 cm,  $P_3$  = R × R of 45 cm,  $P_4$  = R × R of 60 cm, DAS = days after sowing. Values having different letters with same column vary significantly at p = 0.05.

## 3.4. Nutritional Quality Attributes

The research findings depicted the significant influence of genetic divergence and row configuration on the nutritional value of forage cowpea (Figure 4). The protein content (Cp) enhances the nutritional value of forages, and the maximum Cp content was produced by Rawan-2010 sown in a row configuration of 60 cm ( $C_2P_4$ ), while the following treatment combination of  $C_2P_3$  recorded fewer Cp (Figure 4a. The minimum Cp content was recorded for Cowpea–2007 sown in the closer row configuration of 15 cm ( $C_1P_1$ ). Overall, Rawan-2010 outperformed Cowpea–2007, and a row configuration of 60 cm remained unmatched as far as the Cp of forage cowpea was concerned. The increased crude fiber (Cf) content deteriorates the nutritive quality of forages, and Cowpea–2007 remained inferior, especially in a row configuration of 15 cm, by producing the maximized Cf (Figure 4b). Interestingly,

 $C_2P_3$  (Rawan-2010 in 45 cm row spacing) gave the minimum Cf, which was 18% less in comparison to  $C_1P_1$ . This treatment combination was followed by  $C_2P_2$ , which exhibited 11% less Cf content than  $C_1P_1$ . In a similar fashion to Cp, Cowpea-2007 remained inferior to Rawan-2007 under all spatial arrangements by demonstrating increased Cf content. Pertaining to total ash (Ta) content, Rawan-2010 sown in the widest row configuration  $(C_2P_4)$  resulted in the maximum Ta content, and it was followed by the same cultivar sown in the spatial arrangement of 30 cm  $(C_2P_3)$  (Figure 4c). Cowpea-2007 could not perform on par with Rawan-2010, and the minimum value of Ta was exhibited by the closest row spacing of 15 cm, which was 15% less than the most well-performing treatment combination of C<sub>2</sub>P<sub>4</sub>. The research findings of this field trial remained in line with those of Iqbal et al. [22], who opined that leaves are a rich source of crude protein, and a higher leaf number along with a greater leaf area resulted in a significantly higher Cp content of forage soybean. It was also suggested that optimized row arrangement favored nutrient uptake, especially of nitrogen, which boosted amino acid biosynthesis, and ultimately, greater Cp content was recorded, which improved the nutritive value of the forage for dairy animals. Additionally, genetic divergence was also reported to be one of the prime reasons for differences in Cp in forage legume crops, and it was inferred that genetic potential and agronomic package determine the Cp content of forage crops [40,47]. Moreover, it was opined that high planting densities delayed crop switching to reproductive growth, which might be presumably attributed to a higher accumulation of amino acids, which improved Cp content [53]. There exists an inverse relationship between crude fiber content and nutritive quality of forage, as most fractions of fiber are non-digestible by ruminants, and thus, their higher concentration deteriorates feed quality. The lowest quality forage with the maximum fiber content was recorded for the towering type cultivar sown in the narrow row spacing, which was presumably attributed to restricted nutrient supply owing to intense competition for growth resources. Ultimately, higher fiber content was produced. Additionally, the same cultivar had a higher plant height, and owing to its taller stem enriched with fiber, greater fiber contents were recorded. These findings are in agreement with previous studies [47,64,65], whereby taller plants resulted in higher fiber content, and there existed an antagonistic association between crude protein and fiber contents. Similarly, total ash presents the mineral constituents of forages, which are required by animals to maintain the normal functioning of the body. Higher mineral contents improve the nutritive value of feeds [2]. The spreading type cultivar in the widest row configuration resulted in the maximum ash, presumably owing to higher nutrient uptake. These findings corroborate with those of [60,61,63], who opined that row configuration imparted significant influence on mineral constituents of forage crops (both cereals and leguminous forages) through optimization of solar radiation interception and uptake of macro- and micro-nutrients from the soil solution.



Figure 4. Cont.



**Figure 4.** Nutritive quality: (a) protein content, (b) crude fiber content and (c) total ash content of forage cowpea cultivars sown under varying row configurations (details of treatments are presented in the footnote of Tables 1 and 2). Different letters show significant difference at p = 0.05.

#### 3.5. Correlation among Yield Attributes, Seed Yield and Biological Yield

As per correlation analyses, green herbage yield had significant linear relationships with vegetative yield attributes except for the plant height of temperate cowpea cultivars sown under varying configurations. The results indicated a significantly negative association of green herbage yield with plant height ( $R^2 = -0.84^*$ ) (Figure 5a) and a moderately significantly direct relationship with stem girth ( $R^2 = 0.82^*$ ) (Figure 5b) and the number of leaves per plant ( $R^2 = 0.64^*$ ) (Figure 5c). In contrast, leaf area per plant ( $R^2 = 0.89^{**}$ ) of cowpea cultivars sown under different row configurations had a significantly stronger direct relationship with herbage yield (Figure 5d). In a similar fashion, fresh weight per plant of cowpea cultivars ( $R^2 = 0.87^{**}$ ) (Figure 5e) was strongly associated with green herbage yield of cowpea compared to other growth attributes like stem girth and number of leaves per plant. Lastly, correlation analysis of dry weight per plant with dry matter yield of cowpea cultivars sown in different row configurations ( $R^2 = 0.87^{**}$ ) also exhibited a stronger linear association (Figure 4f). These findings pertaining to the negative correlation of plant height with green herbage yield of cowpea cultivars are in contradiction with those of Iqbal et al. [46], who opined that plant height was linearly associated with soybean yield as taller plants assisted in dominating weed populations. Additionally, greater plant height imparted an upper edge to crop plants for up-taking more nutrients from soil solution along with efficient interception of photosynthetically active radiation (PAR) [61,62] compared to many types of indigenous and exotic weeds. Previously, it was reported that stem girth and the number of leaves per plant along with leaf area exhibited stronger

direct associations with yield because the maximum stem girth, being the heaviest part of the plant, contributed significantly towards herbage yield, while greater leaf number and leaf area triggered the photosynthetic rate, which led to maximum biosynthesis of carbohydrates and, ultimately, enhanced yield [54,55,61]. It was also suggested that among vegetative growth traits, leaf area per plant might be used as a reliable indicator for the estimation of crop yield. Likewise, fresh plant weight was directly associated with the green herbage yield of cowpea, indicating higher growth supported by optimized row configuration. Presumably, an improved light environment and use of radiation serve as vital factors enabling plants to attain weight as per genetic potential, which leads to maximized biomass production. It has been suggested that better canopy architecture and greater leaf pigments enhanced intercepted PAR which promoted biomass accumulation by crop plants and thus contributed significantly to boosting crop biomass yields [54]. Moreover, optimized row configurations facilitated positive changes in canopy structure along with photosynthetic capacity, which improved above-ground biomass accumulation, as reported by Xue et al. [54] and Zhang et al. [60]. Furthermore, greater fresh weight per plant resulted in higher dry weight per plant, which was linearly associated with the dry matter yield of cowpea. This might be attributed to better growth and biomass accumulation which increased dry weight per plant and, in turn, increased the dry matter yield of forage legumes as reported by Lithourgidis et al. [63], Ismail and Hall [64], Iqbal et al. [55] and Basaran et al. [53].



Figure 5. Cont.



**Figure 5.** Correlation analyses depicting the relationship between herbage yield and yield attributes such as (**a**) plant height (**a**), stem girth (**b**), leaf area per plant (**c**), number of leaves per plant, (**d**) leaf area per plant, (**e**) fresh weight per plant and (**f**) dry weight per plant. \*\* and \* show highly significant and moderate significant differences at p = 0.05 respectively.

## 4. Conclusions

Here, we have explored the production potential of cowpea to be promoted as an alternative leguminous forage crop in the summer-rainfall environment of the temperate Himalayan region of Pakistan. In this region, farmers and agronomists have been consistently pointing out the dire need for a new competitive forage legume crop in order to provide sustainable supplies of quality forage to dairy animals. From the research findings of this field trial, it might be inferred that cowpea (Rawan-2010 sown in 45 cm row configuration) could potentially serve as a resilient short-duration forage crop having the potential to provide abundant green forage (around 17 t  $ha^{-1}$ ) of superior quality (higher crude protein and total ash content and lower crude fiber content). Additionally, the spreading type of cowpea in a narrow row configuration (30 cm) remained effective in suppressing weed density and their fresh and dry weights, which holds a bright perspective for regions having intensive weed infestations. Moreover, the cultivation package, low requisite mechanization, and profitable farming of numerous food legumes have already been established in the Azad Jammu and Kashmir region of Pakistan; therefore, the expansion of forage cowpea would not pose a challenge, and farmers might conveniently incorporate cowpea in prevalent farming systems of the region. Moreover, the same scenario pertaining to cultivar growth habit and row configuration might be applicable to other cropping areas having similar pedo-climatic conditions globally. Furthermore, the capability of cowpea to withstand the intermittent drought spells and inconsistent rainfall under rainfed conditions in temperate areas needs further field evaluation. Meanwhile, future studies must encompass an evaluation of the economic viability and profitability of forage cowpea compared to other food legumes.

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## Abbreviations

WD	weeds density
PH	plant height
GH	green herbage yield
DM	dry matter biomass
$\mathbf{R}  imes \mathbf{R}$	row to row spacing
RCBD	randomized complete block design
DAP	di-ammonium phosphate
DAS	days after sowing
ANOVA	analysis of variance
СР	crude protein

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