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Adaptive responses of quinoa to diverse agro-ecological environments along an altitudinal gradient in North West Argentina

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ABSTRACT

Quinoa is an important Andean grain crop grown in a wide range of tropical and temperate environments. Time to flowering is an important trait determining grain yield. This work aimed to understand how responses to photoperiod and temperature might alter plant leaf and floral development. To assess the likely degree of G × E interactions, eleven quinoa accessions from a wide range of environments of origin within Northwest Argentina were grown in several sowing dates over two seasons at a high altitude site. In a third season at a low altitude site, a subset of six accessions planted in pots in the field was exposed to two artificially extended and a control (natural) photoperiod. Time to the appearance of floral buds and anthesis were recorded as was leaf number. A photothermal model developed for quinoa was used to compare responses to photoperiod. Plant development rates to visible floral buds and anthesis stages and phyllochron varied widely amongst accessions and across environments within a short day response though phyllochron varied mostly during the reproductive phase. There was a very strong association between time to flowering and altitude of origin ($r = -0.98$), mean temperature of the wettest quarter ($r = 0.98$) and Normalized Difference Vegetation Index values ($r = 0.73$). Photoperiod sensitivity was higher for accessions from the lowlands (normally late flowering), while temperature sensitivity was greatest for accessions from the highlands (early flowering); most variation for these traits detected at the species level was found in North West Argentina. Genotype by environment interactions for yield were related to the traits examined in this study and considering their high heritability it is suggested that quinoa breeding programs targeted for specific adaptation to a wide range of environments can be developed from this germplasm.

1. Introduction

Time to flowering plays a major role in determining grain yield of quinoa across the Northwest Argentina (NWA) region and elsewhere (Bertero et al., 2004; Curti et al., 2014). Consequently, knowledge of the environmental factors that control time to flowering is an essential tool to predict their agro-ecological fit to those environments. Previous research shows that quinoa cultivars adapt to a diverse range of environments through a considerable plasticity in phenology, the main determinants of which are responses to temperature and photoperiod (Bertero et al., 1999a, 2000).

Quinoa cultivars are short-day plants (Bertero et al., 1999b; Christiansen et al., 2010). They vary strongly in their sensitivity to temperature and photoperiod (Bertero et al., 1999a, 2000; Bois et al., 2006). Under photoperiod shorter than a threshold (<11 h), crop duration depends on the cultivar-specific duration of the basic vegetative phase (BVP). If conditions are not inductive however, the BVP is extended by a photoperiod-sensitive phase (PSP) which ends with flower bud initiation. The magnitude of the delay in development beyond the BVP per unit increase of photoperiod (°C day h^{-1}) is termed photoperiod sensitivity (PS) (Bertero et al., 1999a).

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Based on this simple two-phase model Bertero et al. (1999a, 2000) characterized the intraspecific variability in the phyllochron (the interval between the emergence of two successive leaves on the main stem) and flowering responses of quinoa cultivars to temperature and photoperiod. It was shown that both the duration of the BVP (or minimum phyllochron, M_{ph}) and PS for both variables are the main parameters explaining genotypic variability across a latitudinal gradient: genotypes from tropical environments (near the Equator) show high PS and high values for BVP or M_{ph} whereas those from highland (Peru and Bolivia) and sea level origin (Chile) show the lowest values for both parameters (Bertero et al., 1999a, 2000).

According to Bertero (2003), the expected geographical pattern is a longer duration of the BVP and high PS in the presence of a longer growing season, and lower PS under climates characterized by short growing season and/or less inductive photoperiods. The NWA region (as part of the Southern Andean Highlands) offers a "model environment" to test this prediction for quinoa, because the latitudinal and longitudinal gradients in climatic indicators such as reference evapotranspiration, temperature, intra-season dry spell and frost risk determine large differences in the length of the growing season across their agro-ecological zones (François et al., 1999; Garcia et al., 2007; Geerts et al., 2006), and time to flowering is one of the most important variables explaining the genotype-by-environment interaction pattern for yield across genotypic and environmental groups (Curti et al., 2014).

In NWA, Andean farmers sow quinoa cultivars with a wide range of cycle durations. In the highland agro-ecological zone, with a very short growing season, farmers generally sow cultivars with a short cycle (≈90 days from emergence to maturity); while in the humid valleys with a longer growing season cultivars with a longer cycle (≈200 days) are used. Finally in the dry valleys with an intermediate growing season between the highlands and humid valleys, cultivars have intermediate cycle durations (≈180 days) (Curti et al., 2012). This behavior also matches an east-west pattern of altitude increase (and reduction of the mean temperature and rainfall); which determines a progressive shortening of the growing season towards the west (Curti et al., 2012). Consequently, according to Bertero (2003) it can be hypothesized that for quinoa genotypes from NWA the earliness of genotypes from the highlands will be associated with high sensitivity to temperature and lower sensitivity to photoperiod, whereas the opposite will be expected for genotypes from lower altitudes. In this sense, the large variation in phenology of native genotypes from different environments of origin will show a strong fit to specific agro-ecological zones.

To test this hypothesis we conducted field and semi-controlled experiments with photoperiod manipulation with the following objectives: (i) to analyze the effect of sowing dates on responses of time to flowering of a reference set of NWA quinoa genotypes; (ii) to group quinoa genotypes according to their relative responses to sowing dates of time to flowering; (iii) to evaluate photoperiod effects on duration of the emergence-visible floral bud phase and the phyllochron, (iv) to derive response parameters of photoperiod response for both developmental processes and; (v) to examine relationships between photothermal characteristics that determine time to flowering among genotypes and their agro-ecological environment.

2. Materials and methods

2.1. Sowing date experiments

Serial sowing experiments were conducted in Calete, Department of Humahuaca (23° 12′S, 65° 20′W; 2939 masl), province of Jujuy, Argentina, during the 2008–2009 and 2009–2010 growing seasons (austral summer) on a sandy soil (Typic Haplargids, Soil Taxonomy, U.S. Department of Agriculture). Average, maximum and minimum temperatures were obtained from a temperature sensor (TC1047, Microchip Technologies, Chandler AZ) monitored by an automated control unit (Cavadevices, Buenos Aires, Argentina) located in the experimental plots. The study consisted of three sowings for the first (2008–2009) and six for the second (2009–2010) season (Fig. 1) and 11 quinoa genotypes (Table 1). These genotypes were selected from the Faculty of Agronomy of the University of Buenos Aires Germplasm Collection based on their contrasting environments of origin and relative performance for various traits (Curti et al., 2012, 2014). The treatments (sowing dates and genotypes) were arranged in a factorial-nested experimental design, with sowing dates and genotypes as crossed factors randomly assigned to three blocks nested within sowing dates. Plot size was 11 rows (one per genotype) 5 m long, with an inter-row spacing of 0.5 m. Sowing density was 14 seeds m^{-1}, equivalent to 280.000 seeds ha^{-1}.The experiment was kept free of weeds and pests and fertilized at a rate of 43 kg N ha^{-1} 35 days after crop emergence. For all sowing dates experiments received deep irrigation before sowing and then every 10 or 15 days following local practices (furrow irrigation) to avoid water deficits during the crop cycle. Because of the experimental design, all genotypes were exposed to the same irrigation regime. After crop emergence, 10–15 plants were marked in each row × replicate combination and observed at weekly intervals to determine the date of anthesis (time at which

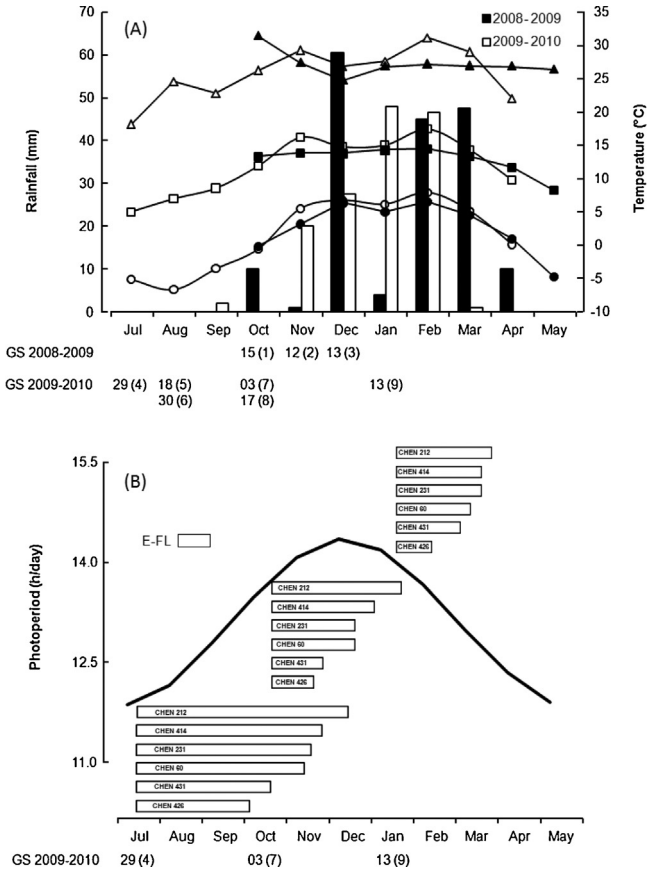

Fig. 1. Climatic conditions for the sowing date experiments in NWA. (A) (○, ●) minimum, (□, ■) mean, (▲, Δ) maximum temperatures (°C) and rainfall (mm, bars) during the 2008–2009 and 2009–2010 growing seasons. Values under the *x* axis indicate the date of sowing and its order (number between brackets) for each growing season (GS). (B) Photoperiod variation (hours) across the GS (continuous line) and emergence (E) to flowering (FL) durations (horizontal bars) for six genotypes sown at three different dates: early (July 2009, identified as 29(4) in Fig. A), conventional (October 2009; 03(7) in A) and late (January 2010; 13(9) in A).

Table 1
Passport data and adaptation group for the genotypes evaluated across sowing dates experiments and artificial daylength extensions under semi-controlled conditions.

Genotype	Origin (location, department, province)	Longitude (O)	Latitude (S)	Altitude (masl)	Genotype group[a]	NDVI[c]	MTWQ (°C)[d]
CHEN 58	Coctaca, Humahuaca (Jujuy)	65° 17′	23° 09′	3215	Dry valley		
CHEN 60[b]	Abralaite de Colanzulí, Iruya (Salta)	65° 14′	22° 54′	3711	Dry valley	0.19	12.8
CHEN 182	QQ 95-NSL 106394, Humahuaca (Jujuy)	65° 20′	23° 12′	2939	Dry valley		
CHEN 212[b]	San Felipe, Santa Victoria Oeste (Salta)	64° 58′	22° 16′	2507	Humid valley	0.34	18.3
CHEN 231[b]	Ocumaso, Humahuaca (Jujuy)	65° 15′	23° 12′	3000	Dry valley	0.25	13.6
CHEN 252	Maimará, Tilcara (Jujuy)	65° 24′	23° 37′	2334	Dry valley		
CHEN 414[b]	La Poma, La Poma (Salta)	66° 11′	24° 42′	3016	Dry valley	0.13	14.4
CHEN 420	Antofallita, Los Andes (Salta)	67° 31′	25° 14′	3498	Highland		
CHEN 426[b]	Santa Rosa de los Pastos Grandes, Los Andes (Salta)	66° 40′	24° 28′	3939	Highland	0.11	10.4
CHEN 431[b]	Susques, Susques (Jujuy)	66° 22′	23° 23′	3619	Highland	0.15	12.6
CHEN 435	Cangrejillos, Yavi (Jujuy)	65° 35′	22° 25′	3583	Dry valley		

[a] Genotype group identified by hierarchical agglomerative clustering of morpho-phenological traits (Curti et al., 2012).
[b] Genotypes evaluated in artificial daylength extensions experiments.
[c] Normalized Difference Vegetation Index (NDVI) dataset with 250 m^2 spatial resolution for the 2000–2013 period.
[d] Mean Temperature of the Wettest Quarter (MTWQ) of the place of origin of genotypes obtained from BIOCLIM data base.

50% of the plants had at least one flower opened (Bertero and Ruiz, 2008)).

2.2. Artificially extended photoperiod experiments

This field experiment was conducted at the Faculty of Agronomy of the University of Buenos Aires (34° 35′S, 59° 29′W; 20 masl) between September 2012 and January 2013. Rainfall and average daily mean temperature were obtained from a meteorological station located 10 m away from the experimental site and for the complete period of the experiment values were 500 mm and 21 °C, respectively. The study consisted of the factorial combination of three photoperiod treatments and six genotypes (Table 1). Treatments were arranged in a split-plot design with photoperiod as the main plot and genotypes as sub-plots. The genotypes selected were CHEN 60, 212, 231, 414, 426 and 431, a subset of those described in Table 1. The geographic range of origin of the genotypes covered the distribution of the crop in the NWA region, showing contrasting latitudes (between 22° 16′ and 24° 42° S), longitudes (between 64° 48′ and 65° 40′W), altitudes (between 2334 and 3939 masl), precipitation and temperatures (decreasing in the east–west direction) (Curti et al., 2012). The natural season photoperiod (PP_N) varied from 12.0 h for the early-flowering genotype (CHEN 426) to 12.4 h for the late-flowering genotype (CHEN 212) for the period between emergence and visible floral buds. Where longer daylengths were imposed the natural daylength was extended to 15 (PP_{15}) and 18 h (PP_{18}). Photoperiodic extensions were performed by using a combination of lamps (a set of two 40 W incandescent bulbs and one 36 W fluorescent tube per sub-plot, adding 0.6 and 0.97 mol of PAR $m^{-2}\,d^{-1}$ according to photoperiodic treatment) which were programmed to automatically turn on and off by an electronic timer. This combination of incandescent and fluorescent lamps provided a light quality similar to natural radiation (red:far red quantum ratio of 1.17). Artificial daylength extensions were performed before dawn and after dusk, from 0530 to 0730 h and 1730 to 2030 h for PP_{15}, and from 0400 to 0730 h and 1730 to 2200 h for PP_{18}, respectively. For each genotype, the lamps were regularly elevated to maintain in them 50 cm above the plant canopy as it grew. Each photoperiod treatment included 60 pots (combination of six genotypes and 10 pots per genotype) covering an area of ~10 square meters and were 5 m away from each other in order to prevent the exposure of plants to light from neighbouring treatments. The experiment was conducted on seven-litre capacity pots filled with 25% perlite, 25% peat and 50% of local soil. Five seeds were sown per pot, and thinned to one plant per pot after crop establishment (10 plants per treatment). Density was 25 plants m^{-2} and pots were randomly rearranged every week to minimize edge effects. Nitrogen application consisted of 0.6 g per pot (equivalent to 70 kg N ha^{-1}) applied 11 days after emergence, and again at day 33 due to chlorosis signs associated with an excess in rainfall. Cypermetrine (Syngenta), Chlorpyrifos (Syngenta) and Azoxystrobin + Cyproconazole (Amistar, Syngenta) were applied to prevent pest and fungal diseases. Plants were hand-weeded and watered as needed. Time to visible floral bud stage (estimated as visible inflorescence primordia) and 1 stanthesis were recorded (Bertero and Ruiz, 2008). The main stem leaf number was counted every 2–3 days for all plants per genotype per treatment combinations. Leaves were counted when their length exceeded 1 cm. Leaf count was followed up to stability of leaf number. In this study data to VFB are used for most analyses, since the responses observed were similar for both phases.

2.3. Statistical analysis

2.3.1. Sowing date experiments

A combined analysis of variance was performed to investigate the effects of genotype (G), sowing date (S) and their interaction (G × S) on time to flowering (days). The components of variance were computed using the Type III sum of squares since some blocks were lost. To investigate similarities in terms of genotype-specific responses of time to flowering across the range of sowing dates, a hierarchical agglomerative clustering method with incremental sum of squares as fusion criterion was utilized. The squared Euclidean distance was used as the dissimilarity measure for Ward's method. Then a multivariate analysis of variance was performed to define the optimal number of groups and a one-way analysis of variance to test for statistical differences among the recognized groups.

2.3.2. Artificial daylength extension experiments

The duration of the vegetative phase (from emergence to visible floral buds, hereafter E-VFB) was expressed in thermal time units (T_b = 3 °C) (Bertero et al., 1999a). Thermal time sums were calculated by subtracting the base temperature from the mean daily temperature for the E-VFB phase. The period of leaf appearance was divided into two sub-periods: (1) from crop emergence to the 1st pair of visible leaves; and (2) from the first pair of visible leaves to the end of leaf appearance (ca. anthesis) (Bertero, 2001). Thermal time accumulation for leaf appearance was calculated using a base temperature of 2 °C (Bertero et al., 2000). Rates of leaf appearance were calculated for the second sub-period as the slope of the relationship between the number of visible leaves and thermal time from emergence using the bi-linear model:

$$LN = a + b\mathrm{TT_{ap}} \text{ if } \mathrm{TT_{ap}} < c \quad (1)$$

$$LN = a + b\text{c if } \mathrm{TT_{ap}} > c \quad (2)$$

where LN is leaf number, TT_{ap} the cumulative thermal time (°Cd, T_b = 2 °C) from emergence to the end of leaf appearance (ca. anthesis), *a* and *b* the slope and intercept of the linear regression and *c* the thermal time value at which leaf appearance ceases (plateau). Phyllochrons were calculated as the reciprocal of the rate of leaf appearance from Eq. (2) (Bertero et al., 2000). Analyses of variance were conducted to evaluate the impact of photoperiod treatment (P), genotypes (G) and their interaction (P × G) on duration of the E-VFB phase and phyllochron according to a complete randomized split-plot design.

The Major and Kiniry (1991) model adapted to quinoa (Bertero et al., 1999a, 2000) was used to characterize photoperiodic responses for duration of the E-VFB phase and the phyllochron. Since there were only three photoperiod treatments, the dataset from this experiment did not allow adjusting models of increasing complexity for photoperiodic responses. Therefore, and to derive the PS parameter for both development processes, duration of the E-VFB phase and phyllochron values were plotted as a function of photoperiods (mean value for the entire period of analysis for control treatment) explored for each combination of photoperiod and genotype treatments. PS was estimated as the slope of the linear regression fitted. On the other hand, since a minimum duration of E-VFB phase and phyllochron was not detected, duration for both development processes under PP_N was used as a proxy of their values.

2.3.3. Relationships between photothermal characteristics and environment of origin

To characterize the agro-ecological environment we used indices including latitude, longitude, altitude, mean temperature of the wettest quarter (i.e., Austral summer) and the Normalized Difference Vegetation Index (NDVI). The integral of NDVI index across the growing season strongly correlates with aboveground net primary productivity and time from emergence to flowering is the main mechanism by which genotypes can adjust to variation in environmentís availability of resources. The MODIS 16-day composite NDVI dataset with 250 m^2 spatial resolution for the 2000–2013 period was used in the study (the MOD13Q1 product). Using the coordinates for each genotype, the NDVI data for the period was downloaded from http://daac.ornl.gov/MODIS/modis.shtml. For CHEN 426 those pixels included a meadow, and were displaced a few seconds in latitude and longitude to a closer plain. Then, the integral of NDVI was calculated as the mean of values of the growing season for the 2000–2013 period in cultivated plots. The mean temperature of the wettest quarter was collected from the global climate grid layers provided by WorldClim (www.worldclim.org) with a resolution of 0.0083° (≈0.88 ± 0.01 km). To quantify the significance and magnitude of the relationships between genotypeís photothermal characteristics and their agro-ecological environment, *t* test and Pearson correlations were used. Statistical analyses were carried out using the Infostat package (Di Rienzo et al., 2011).

3. Results

3.1. Variability of environmental conditions and crop duration

Weather conditions are shown in Fig. 1. Average, maximum and minimum temperatures were similar among years between October and April (Fig. 1A). The most important difference between years was related to rainfall accumulation and distribution. The 2008–2009 growing season presented higher precipitation values (178 mm) and a more heterogeneous distribution than 2009–2010, which showed lower values (145 mm) and a more homogeneous distribution (Fig. 1A). The highest maximum temperatures were registered in October for 2008–2009, and during November and February for 2009–2010 (Fig. 1A), due principally to high cloudiness in months of higher radiation. The photoperiod and temperature ranges explored during the second growing season were higher by including a wider range of sowing dates starting in July (Fig. 1A and B); for this season the lower maximum and minimum temperatures were registered during July, August and September and the highest thermal amplitude during August (Fig. 1A).

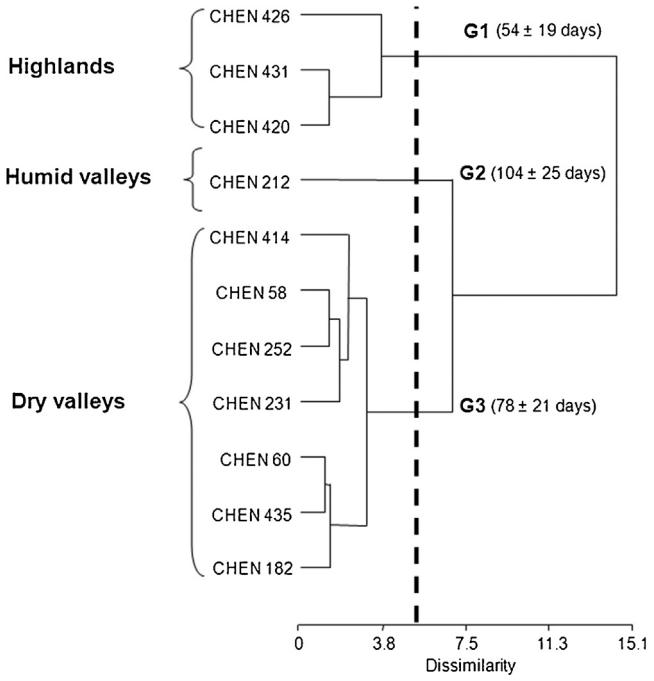

Fig. 2. Hierarchical classification of 11 genotypes of quinoa according to their relative responses for time to flowering across nine sowing dates. G = genotypic group. Values between parentheses are mean duration to flowering (±1 standard error) for each group across sowing dates.

Combined analysis of variance revealed significant S, G and G × S interaction effects for time to flowering. The S term accounted for a higher proportion of variation followed by G and G × S interaction effects (data not shown). The ratio among sources of variation for G and G × S interaction was 7.4:1. Durations to flowering were highest for the July and August sowing dates, followed by the conventional sowing dates(October) and shortest at the later sowings (November, December and January). This pattern is shown in an example for three contrasting sowing dates (Fig. 1B). Genotypes showed larger differences in time to flowering at the earlier and conventional sowing dates as compared with later sowings (Fig. 1B).

Cluster analysis showed that genotypes were grouped into three groups with different response patterns across sowing dates (Fig. 2). Group 1 consisted of three genotypes (CHEN 420, 426 and 431) previously assigned to Highlands (Curti et al., 2012) that showed, across-sowings and compared with other genotypic groups, shorter time to flowering (54 ± 19 days) and smaller relative variation in the length of the pre-flowering period (see Fig. 1B for response patterns across sowing dates for genotypes CHEN 426 and 431). Within this group, genotype CHEN 426 showed the shortest duration (44 ± 18 days) compared with CHEN 420 and 431 (58 ± 19 days), however no significant differences were detected between all three genotypes. Group 2 consisted of one genotype (CHEN 212) previously assigned to Humid Valleys (Curti et al., 2012), that showed the highest duration of time to flowering for all sowings (104 ± 25 days) and the largest relative variation in the length of the pre-flowering period (Fig. 1B). The last group

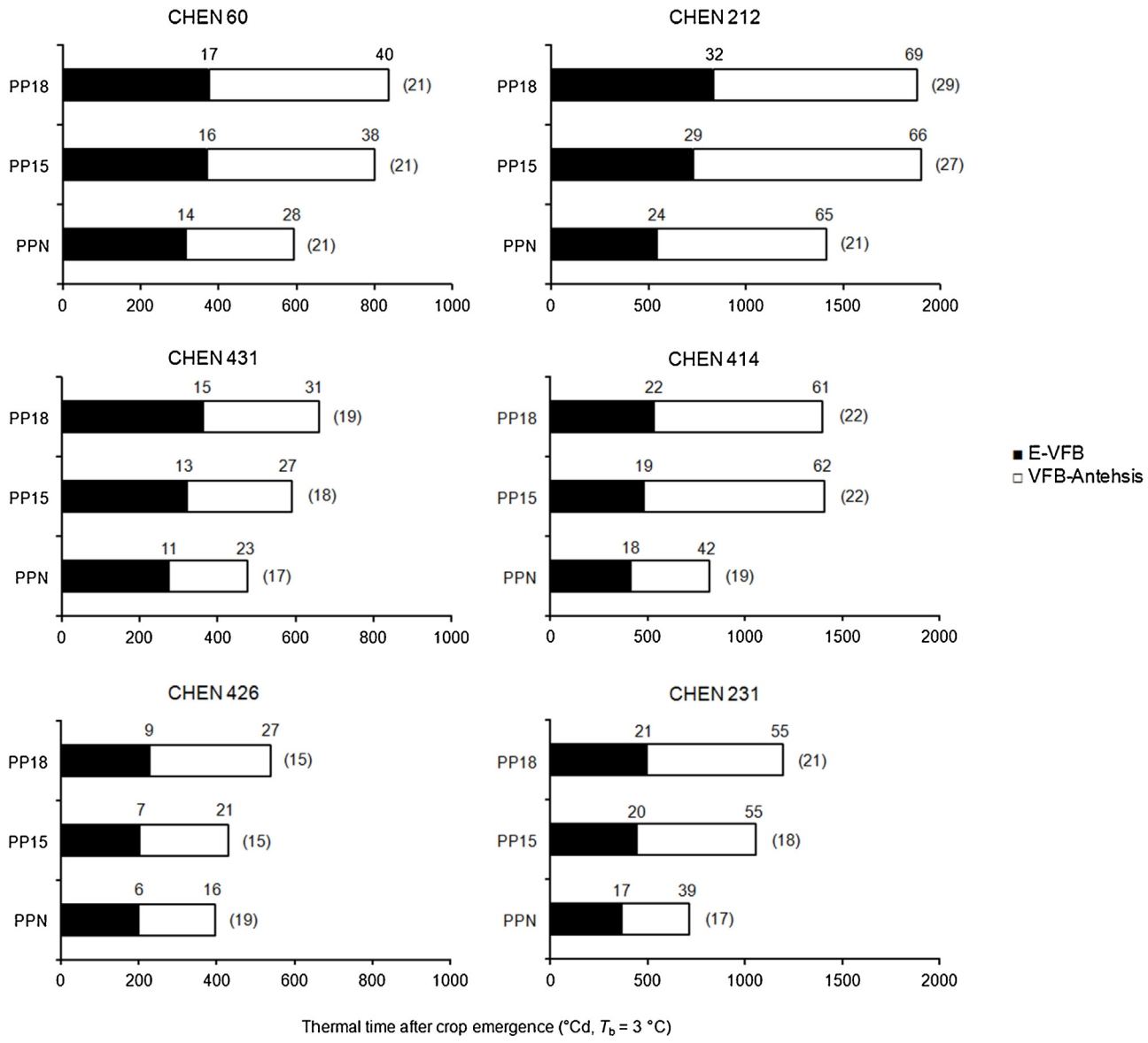

Fig. 3. Duration in thermal time of different developmental phases in six quinoa genotypes under three photoperiodic conditions: (PP_N) natural photoperiod, (PP_{15}) photoperiod extension to 15 h and (PP_{18}) photoperiod extension to 18 h. Phases are: (■) plant emergence (E) to visible floral buds (VFB) and (□) VFB to anthesis. Numbers above bars indicate the number of leaves at the end of each developmental stage. Numbers in brackets at the right of bars indicates the average phyllochron values for each photoperiodic treatment calculated on the basis of T_b = 2 °C for the period between the appearance of the first pair of true leaves and anthesis. The *x* axis scales differ between the left and right hand figures.

to join the cluster (G3), consisted of seven genotypes (CHEN 58, 60, 182, 231, 252, 414 and 435) previously assigned to Dry Valleys (Curti et al., 2012), that showed intermediate durations to flowering (78 ± 21 days) and higher variation in the length of the pre-flowering period compared with G1 (see CHEN 60, 231 and 414in Fig. 1B).

A preliminary analysis was conducted to describe the photoperiod response of time to flowering (expressed as thermal time, with a T_b = 3 °C) as a function of photoperiodic conditions explored in the field using the model proposed by Bertero (2003). Results of this analysis did not show a clear association (data not shown). This lack of association could be due either to the range of photoperiods explored, the strong co-variation in temperature and photoperiod or that other environmental factors such as incident radiation could have modified the development rate to flowering.

3.2. Photoperiod effects on the E-VFB phase and the phyllochron in the study using photoperiod extensions

There was a significant effect of daylength extensions on the duration of E-VFB phase for all genotypes. A quantitative short-day response was observed as the E-VFB phase was extended by daylength treatments (Fig. 3) and no change in ranking was observed among genotypes between photoperiod treatments.

All genotypes showed positive values for the photoperiod sensitivity parameter (Table 2). Both photoperiod sensitivity and minimum E-VFB duration under PP_N showed a wide range of variation among genotypes from different places of origin (Table 2). Genotypes with long duration of the E-VFB phase under PP_N (CHEN 212, 231 and 414) have higher values of photoperiod sensitivity. Temperature sensitivity, calculated as the inverse of the duration of E-VFB phase under PP_N ($°Cd^{-1}$) exhibited a consistent and inverse relationship with photoperiod sensitivity ($r = -0.83$, $P < 0.001$). This means that genotypes with high sensitivity to temperature have

Table 2
Parameters characterizing the response of the duration of E-VFB phase and phyllochron to photoperiod. M_D and M_{ph} means the minimum duration of E-VFB phase and phyllochron, respectively.

Genotypes	E-VFB phase (T_b = 3 °C)		Phyllochron (T_b = 2 °C)	
	M_D	Model (°Cd)[a]	M_{ph}	Model (°Cd)[a]
CHEN 60	315	TT = 197.3 + 10.3 P	21	TT = 21
CHEN 212	543	TT = 188.5 + 30 P	21	TT = 4.3 + 1.41 P
CHEN 231	367	TT = 93.1 + 22.8 P	17	TT = 8.2 + 0.69 P
CHEN 414	413	TT = 155.9 + 21.2 P	19	TT = 13.2 + 0.51 P
CHEN 426	199	TT = 139.3 + 4.7 P	19	TT = 26.3 − 0.67 P
CHEN 431	274	TT = 188.5 + 15 P	17	TT = 12.9 + 0.34 P

[a] For all regressions the coefficients of determination were ≥ 0.90 ($P < 0.01$).

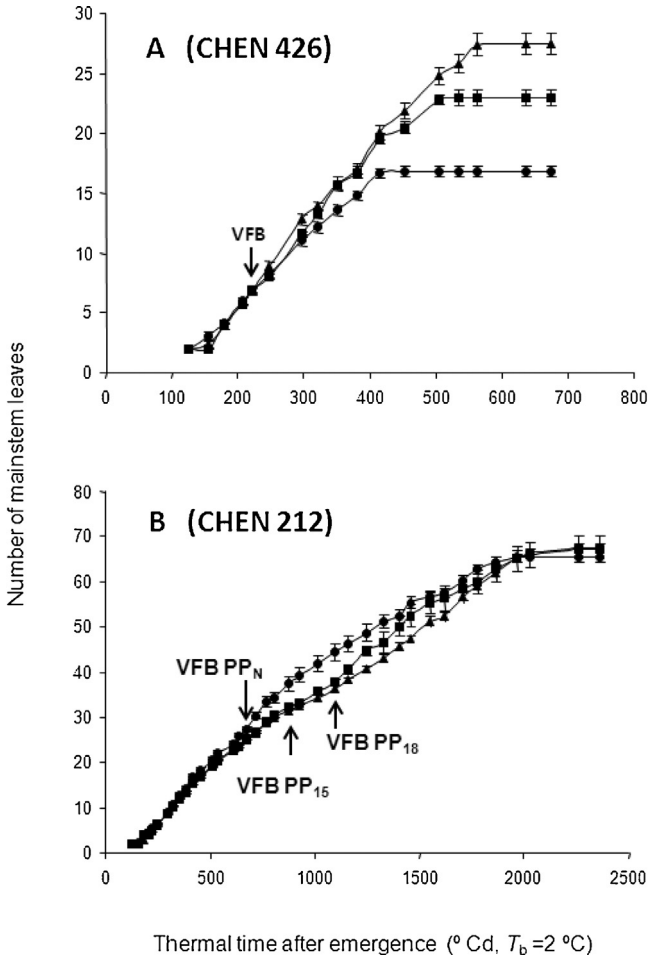

Fig. 4. Number of expanded leaves under three photoperiodic conditions: (●) natural photoperiod (PP_N), (■) photoperiod extension to 15 hours (PP_{15}) and (▲) to 18 h (PP_{18}). Arrows indicate the date of VFB stage under different photoperiodic treatment. Vertical bars in each symbol represent the standard error of the mean.

low photoperiod sensitivity and vice versa. Similar results were observed for the reproductive phase (VFB-anthesis) and sensitivity to photoperiod was more pronounced during this period. It varied from 18 to 81.8 °Cd h^{-1} as compared with 4.7–30 °Cd h^{-1} for the E-VFB phase (data not shown).

A single value of 95 °Cd was calculated as the thermal time accumulation for the duration of the first sub-period of leaf appearance. Then, there was a linear relationship between the number of visible leaves and thermal time for the second sub-period (Fig. 4). The rate of leaf appearance decreased during the reproductive phase in plants exposed to long days (PP_{15} and PP_{18}). Rates of leaf appearance were higher under PP_N in four out of six genotypes; exceptions were CHEN 60 which did not show differences between photoperiods, and CHEN 426 in which the rate of leaf appearance increased under long days (Fig. 4A).

In all other combinations, the rate of leaf appearance decreased with increasing photoperiod mainly after VFB stage, and this caused leaf number under long days to overcome that under PP_N only near the end of the reproductive phase. Despite this complexity, there was a strong and positive relationship between the reproductive dynamics (i.e., duration to VFB and anthesis) and the number of visible leaves at the end of both stages ($r = 0.98$ and $r = 0.99$, $P < 0.001$ respectively). An exception, however, was CHEN 212 in which a longer duration of the reproductive phase did not compensate for the impact of photoperiod in decreasing the rate of leaf appearance. Moreover, for this genotype a similar final number of leaves was reached in all treatments (Fig. 3 and 4B).

In accordance with the effect of photoperiod on the rate of leaf appearance, the phyllochron increased in four out of six genotypes under long days (exceptions were CHEN 60 and 426 as mentioned) (Fig. 3). The large phyllochron values observed for CHEN 426 under PP_N was a consequence of fast development to flowering, so that there was no time for acceleration of leaf appearance later in development. Clearly, the dynamics of progression of leaf appearance was more complex than just a constant phyllochron. During the first development phase, daylength extension did not lead to large differences in the dynamics of leaf appearance compared with behaviour during the reproductive phase (Fig. 4B). It is during this latter phase that all genotypes expanded most leaves (Fig. 3). Even in genotypes that did not show differences in the phyllochron like CHEN 60, the number of visible leaves during the reproductive phase increased under long days (Fig. 3) and this effect was mainly due to a longer duration of the reproductive phase in long days (Fig. 3). Because of photoperiod effects on the phyllochron duration of the reproductive phase and the number of visible leaves expanded during the phase, it exhibited a less strong association ($r = 0.93$, $P < 0.001$) than during the E-VFB phase.

A linear relationship adequately described average phyllochron responses to photoperiod and this information is summarized in Table 2. Four out of six genotypes exhibited a significant quantitative short-day response for this trait, with genotypes from Dry and Humid Valley being more sensitive than those from Highlands.

3.3. Association between traits

There was a negative relationship between the altitude of origin of genotypes and the minimum duration of the E-VFB phase ($r = -0.98$) as well as with photoperiod sensitivity ($r = -0.96$). Consequently, genotypes cultivated at higher altitudes (Highlands, Table 1) showed low photoperiod sensitivity and shorter minimum duration of the E-VFB phase compared to those from lower altitudes (Dry and Humid Valleys genotypes) (Table 2). Besides that, there was a positive relationship ($r = 0.73$) between the length of the E-VFB phase under PP_N and NDVI of the place of origin of genotypes (Fig. 5). Moreover, the minimum length of the E-VFB phase and sensitivity to photoperiod for this phase were positively associated with mean temperature of the wettest quarter ($r = 0.98$ and 0.92, respectively). For the phyllochron, there was a negative relationship between its photoperiod sensitivity and altitude of origin of genotypes ($r = -0.94$), and a positive association with the mean temperature of the wettest quarter ($r = 0.95$), the minimum duration ($r = 0.93$) and sensitivity to photoperiod for the E-VFB phase ($r = 0.98$), respectively.

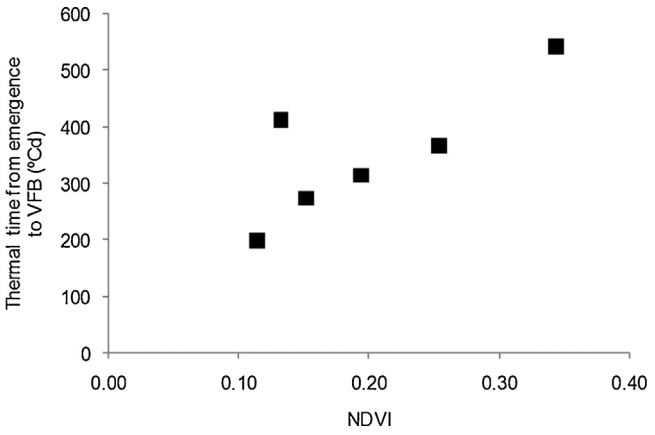

Fig. 5. Relationship between the minimum E-VFB phase and Normalized Difference Vegetation Index (NDVI) of the place of origin of genotypes.

4. Discussion

4.1. Genotypic variation in responses to photoperiod and temperature and their adaptive importance

According to our results, there was large variation in time to flowering among the 11 quinoa accessions when grown under both time of planting and modified photoperiod studies. Much of this variation was due to large genotypic effects. This strong genotypic control suggests that achieving a high response to selection for time to flowering, and selection of genotypes with contrasting patterns of adaptation to different environment and sowing dates should be feasible.

The response of quinoa genotypes for time to flowering were organized into three groups linked to altitude of origin and type of environments: Highlands (G1), Humid Valleys (G2) and Dry Valleys (G3) (Fig. 2). When a sub-set of these genotypes were grown under different photoperiods large genotypic variation in responses to temperature and photoperiod for development rate to flowering and phyllochron was found across groups (Fig. 3, Table 2). In previous studies considerable variation in minimum duration of the E-VFB phase (310–639 °Cd), sensitivity to photoperiod (12–59.7 °Cd h^{-1}) and sensitivity to photoperiod for the phyllochron (0.03–1.6 °Cd h^{-1}) was observed among cultivars from a very large latitudinal range. Those from low latitudes were more sensitive to photoperiod and with longer minimum E-VFB duration or M_{ph} compared to those from the southern Bolivian highlands and sea level (Bertero et al., 1999a, 2000). The ranges of variation in sensitivity to photoperiod and minimum E-VFB duration detected for NWA represents 53% and 100% of that found at the species level (Table 2, Bertero et al., 1999a), and 100%and 57% of that for phyllochron sensitivity to photoperiod and M_{ph}, respectively (Table 2, Bertero et al., 2000). The combination of traits determining variation in development across a wide latitudinal range and within a narrow latitudinal range but a high altitudinal range, as sampled in this study, are the same. This exemplifies how a large environmental variability found in a small geographic range promotes a high phenological variability.

How does genotypic variation in responses to temperature and photoperiod found within this germoplasm regulate adaptation of quinoa genotypes to diverse agro-ecological environments in NWA? Genotypic differences in time to flowering contribute to structuring G × E interaction for grain yield and determine the agro-ecological fit of genotypes to different group of environments in NWA (Curti et al., 2014). Variation in responses to both temperature and photoperiod found in the present study is the underlying cause determining those differences in phenology among genotypes and its adaptive significance is suggested by the strong association found between the photothermal characteristics of genotypes and variables characterizing their environments of origin.

Earliness was determined by higher sensitivity to temperature and low sensitivity to photoperiod of development rate to flowering (E-VFB^{-1}) and was associated with climates with low NDVI and lower mean temperatures during the crop cycle, namely with Highlands environments characterized by drought stress or high frost risk (François et al., 1999; Garcia et al., 2007; Geerts et al., 2006). In these environments, cropping calendar options are restricted by a very short length of the growing season and require short duration genotypes which escape from terminal drought and early frost (Curti et al., 2012; Garcia et al., 2007). These genotypes show higher relative yield associated with high conversion of biomass to grain (Curti et al., 2014). Conversely, these genotypes are not adapted to Humid and Dry Valley sites, showing a high development rate in environments characterized by higher mean temperature during the crop cycle, and paying a cost in term of yield potential due to the shorter available time to capture resources (Curti et al., 2014).

Lateness was determined by higher sensitivity to photoperiod and lower sensitivity to temperature and was associated with climates with high NDVI and higher mean temperatures during the crop cycle from Humid and Dry Valley sites of lower elevation characterized by longer growing seasons. These genotypes are well adapted since both traits contribute to higher biomass stability at flowering (Giménez et al., 2013) and yield is maximized through a longer time to flowering (Curti et al., 2014). Longer time to flowering limits adaptation to Highland environments because of their higher risk of facing terminal drought and/or early frost (Curti et al., 2014) however.

The gradients in developmental responses to photoperiod and temperature explored in this study were analyzed considering their adaptive role to environments characterized by differences in crop season length. Variation in temperature sensitivity agrees with Atkinson and Porter (2016) who stated that, in environments characterized by drought stress and frost risk at the end of the growing season, a developmental rate highly sensitive to temperature enable the crop to produce grain through earlier flowering and maturity whereas in warmer environments, a reduced sensitivity to temperature might help increase yields, by preventing unnecessary shortening of the growing period. As a short day species originating in the tropics, quinoa compares with species from similar origin. Adaptation to highland environments is regulated by a shorter BVP and lower sensitivity to photoperiod for time to flowering in maize whereas the opposite combination is found in lowlands populations (Gouesnard et al., 2002). Upland-adapted japonica rice cultivars escape terminal drought stress by combining a short BVP and low photoperiod sensitivity (Dingkuhn and Asch 1999). In sorghum, earliness in combination with low sensitivity to photoperiod determine adaptation to high latitudes sites (Kouressy et al., 2008) and a long BVP plus high sensitivity to photoperiod contributes to homeostasis of flowering time at given locality in low latitudes sites characterized by long growing season (Craufurd et al., 1999; Craufurd and Qi, 2001).

4.2. Leaf number, phyllochron and reproductive phase duration

Reproductive phase (VFB to anthesis) duration was prolonged by photoperiod extensions in two ways: by affecting the number of leaves expanded during it (all genotypes) or by increases in the phyllochron (four genotypes) (Fig. 3). It is known for quinoa that the total number of leaves is the consequence of the number of primordia initiated during the vegetative phase but can also respond to direct photoperiod effects exerted during the reproductive phase via effects on the proportion of primordia expanded during it; some

will become bracts under short days or expanded leaves under long days (Bertero et al., 1999b).

Photoperiod effects on the phyllochron affecting duration of the reproductive phase are reported for other crops, i.e. in sorghum (Clerget et al., 2008), barley and wheat (Miralles and Richards, 2000). For leaf number even in determinate crops like wheatyoung primordia are labile and can become either spikelets or leaves if plants are shifted to more or less inductive conditions (Rawson and Zajac, 1993) and final commitment of the first spikelet primordia was reported to occur as late as the terminal spikelet stage in some genotypes (Brooking et al., 1995). Exploitation of knowledge of the genetic and environmental determinants of plasticity in duration of the reproductive phase as a way to guide breeding of major field crops has been extensively analysed (see revision in Slafer et al., 2009); however more detailed analysis of the interactions between photoperiod and other factors like temperature affecting primordia plasticity in quinoa are needed.

An important consequence of photoperiod sensitivity during the reproductive phase is that other simultaneous processes are affected as a result of its prolonged duration under long days. A longer duration can affect key process of yield determination such as the generation of reproductive structures (as seen in other grain crops, Slafer et al., 2009) and leaf area (Ruiz and Bertero, 2008). Because differences in the phyllochron largely occur during the last part of the period of leaf appearance (reproductive phase) and there is an important overlapping between leaf growth and reproductive development (Bertero, 2015), sensitivity to photoperiod of this phase may increase the duration of the period of generation of reproductive structures which are linked with yield via both changes in the phyllochron and the number of leaves. Moreover an increase in the amount of radiation interception around the critical period of yield determination could be gained as was reported for other grain crops (Andrade et al., 2005; Miralles et al., 2000; Slafer et al., 2001).

5. Conclusions

This study shows how gradients of variability in developmental responses to photoperiod and temperature explain the agro-ecological fit of quinoa genotypes to their environment of origin and how this response affects G × E interaction for grain yield across a highly diverse region as the NWA. This information is useful to guide breeding strategies for different agro-ecological zones in NWA region by designing cultivars adapted to particular environments (narrow adaptation). It also provides a guide to unravelling the genetic basis of adaptation to environments characterized by strong variability in climatic conditions.

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