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Karyological variability and chromosomal asymmetry in highland cultivars of *Chenopodium quinoa* Willd. (Amaranthaceae)

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Abstract. *Chenopodium quinoa* Willd. is rapidly gaining importance worldwide as a superfood. However, structural diversity and asymmetry analyses of chromosomes of different cultivars of the species are largely understudied primarily owing to their small chromosomes. In this paper, karyomorphological investigations were performed on 21 cultivars of *C. quinoa* with varying seed morphology cultivated widely in the highland regions of the Andes, which is the center of domestication of this species. Somatic chromosome number was found to be 2n= 36 in all cultivars with no occurrence of mixoploidy. Lengths of individual chromosomes varied between 0.63–6.53 µm, with their short arms ranging from 0.25–2.95 µm and long arms between 0.38–3.58 µm. Types of primary constriction ranged from median to sub–terminal. One pair of chromosome in each complement possessed a secondary constriction. Chromosome complements of all cultivars belonged to the asymmetry class 2B with an average asymmetry index value of 3.21±0.61. Values of intra- and inter-chromosomal asymmetry indices were 0.30±0.02 and 0.20±0.02 respectively across all cultivars. The average coefficients of variation of chromosome lengths was 19.77±2.11 and average centromeric index was 16.28±2.12. Arm ratio of the chromosomes varied from 0.34 to 5.76. The mean values of karyotypic asymmetry, symmetry index and karyotype asymmetry index percentage were 17.69±1.53, 147.69±5.54 and 58.71±0.86 respectively. Pearson correlation revealed strong correlation within inter– and intra–chromosomal asymmetry indices. Our analyses uncovered higher chromosomal variation in quinoa than previously found with high inter–varietal similarities among the studied cultivars, revealed from scattered diagrams between asymmetry indices.

Keywords: *Chenopodium quinoa*, chromosome, karyotype**,** chromosomal asymmetry, inter-varietal symmetry.

INTRODUCTION

Sustainable agricultural systems generally focus on traditional commercial food crops, which might not be adequate in the near future to fulfill the projected increase in global consumption due to increase in population. This, along with rapid climate change, has necessitated the investigation and

exploitation of alternative crop plants to complement the projected deficit (Graf *et al.* 2015). Recent research has focused on the South American crop *Chenopodium quinoa* (quinoa), which the Food and Agriculture Organization of the United Nations have touted as a crop that can mitigate global food demand to a large extent (Graf *et al.* 2015). This plant's adaptability to extreme agro–ecological conditions together with its balanced proportion of amino acids, carbohydrates, lipids, vitamins and minerals, low glycemic acid content and its gluten–free nature, have resulted in expansion of its cultivation outside its native region (Ruiz *et al.* 2014; Pereira *et al.* 2019). Improvement programs of the crop include reduction in saponin content of its seed coat, increasing compactness of its inflorescence, high stress tolerance and resistance to pests and pathogens (Murphy *et al.* 2016; Jarvis *et al.* 2017). Success of these programs demand identification of existing genetic divergence in the species.

An initial idea of genetic variability among plants can be perceived by analyzing cytological characters (Guerra 2008). Most crop plants have undergone recent polyploidization events (Renny-Byfield and Wendel 2014). Polyploidy results in variable chromosome numbers and the ensuing chromosomal changes like translocations and inversions give rise to structural variabilities (Leitch and Leitch 2008). Analyses and comparison of these 2 parameters, viz. chromosome number and structure, among related taxa is considered to be a reliable approach for quantitative interpretation of their similarity or divergence among related plants (Bennett and Leitch 2005). Generally considered an allotetraploid with a basic chromosome number x= 9 (Krak *et al.* 2016; Mandák *et al.* 2016), the reported somatic chromosome counts of quinoa predominantly showed an invariable 2n= 36 (Kjellmark 1934; Wulff 1936; Cárdenas and Hawkes 1948; Heiser 1963; Gandarillas and Luizaga 1967; Giusti 1970; Kolano *et al.* 2001, 2012; Rahiminejad and Gornall 2004; Bhargava *et al.* 2006; Palomino *et al.* 2008; Mandák *et al.* 2016). However, there are limited studies on variations in chromosome structure and asymmetry in the species mainly due to their small size, which makes common karyotyping of the species difficult (Kolano *et al.* 2001, 2012). Such studies, when present, were mostly limited to examining only 1 or 2 varieties restricting the possibility of understanding inter-varietal chromosomal differences, or, were based on assessments of insufficient asymmetry parameters (Kolano *et al.* 2001, 2012; Bhargava *et al.* 2006; Maughan *et al.* 2006; Palomino *et al.* 2008; Yangquanwei *et al.* 2013).

Earliest reports of domestication and cultivation of quinoa can be traced back to about 7,000 years ago to the Inca dynasty in South American Andes, especially near Lake Titicaca from Peru and Bolivia that constituted the ancestral highland ecotype of this species (Jarvis *et al.* 2017). Although variations in prominent morphological markers for commercial quinoa like seed size and colour, exist within the cultivated varieties of South America, it can be argued that, prolonged farming of a species at and around its center of domestication and diversity might expose them to the risk of genetic erosion, resulting in low genetic variability (Gonzalo *et al.* 2019). Existence of such genetic erosion, undesirable for germplasm improvement and conservation programmes, might be realized from cytological studies; however, chromosomal study of quinoa from this region is not forthcoming.

The objectives of our work were (1) to analyze the karyotype structure of highland varieties of quinoa, (2) to determine the extent of karyotype differentiation among the varieties, (3) to evaluate chromosomal asymmetry in the varieties. We investigated 21 morphologically variable highland cultivars of quinoa, and, performed karyotypic analyses based on asymmetry parameters that could help in elucidating worthwhile information on the inter–varietal divergence of quinoa.

MATERIALS AND METHODS

Materials

For the purpose of this study, seeds of white, yellow, red and black–coloured varieties were selected from the highland ecotypes of quinoa, their sizes ranging from 1.5 to 3.0 cm in diameter. These cultivars were collected from Peru and Bolivia around Lake Titicaca (Figure 1). Seeds from at least 5 individuals of each cultivar were germinated on moist filter paper in Petri plates, kept in dark for 24–48 h at room temperature for cytological study. Growing root tips of freshly germinated seedlings were used for cytological analyses.

Methods

Actively growing 1.5–2 cm long root tips of seedlings were harvested after 1 to 2 days of germination initiation, between 10 AM to 11 AM, for mitotic study. Pretreatment of the harvested root tips were performed in aqueous solution of 2 mM 8–hydroxyquinoline for 3 hours at 10°C after which the root tips were fixed overnight in Carnoy's fixative at 10°C. Hydrolysis, staining of the root tips and slide preparation were done following the protocol of Sun *et al.* (2017). Squashed root tips were observed under Olympus BX51 microscope (Olympus, Germany) at a magnification of 1000X. Well scattered

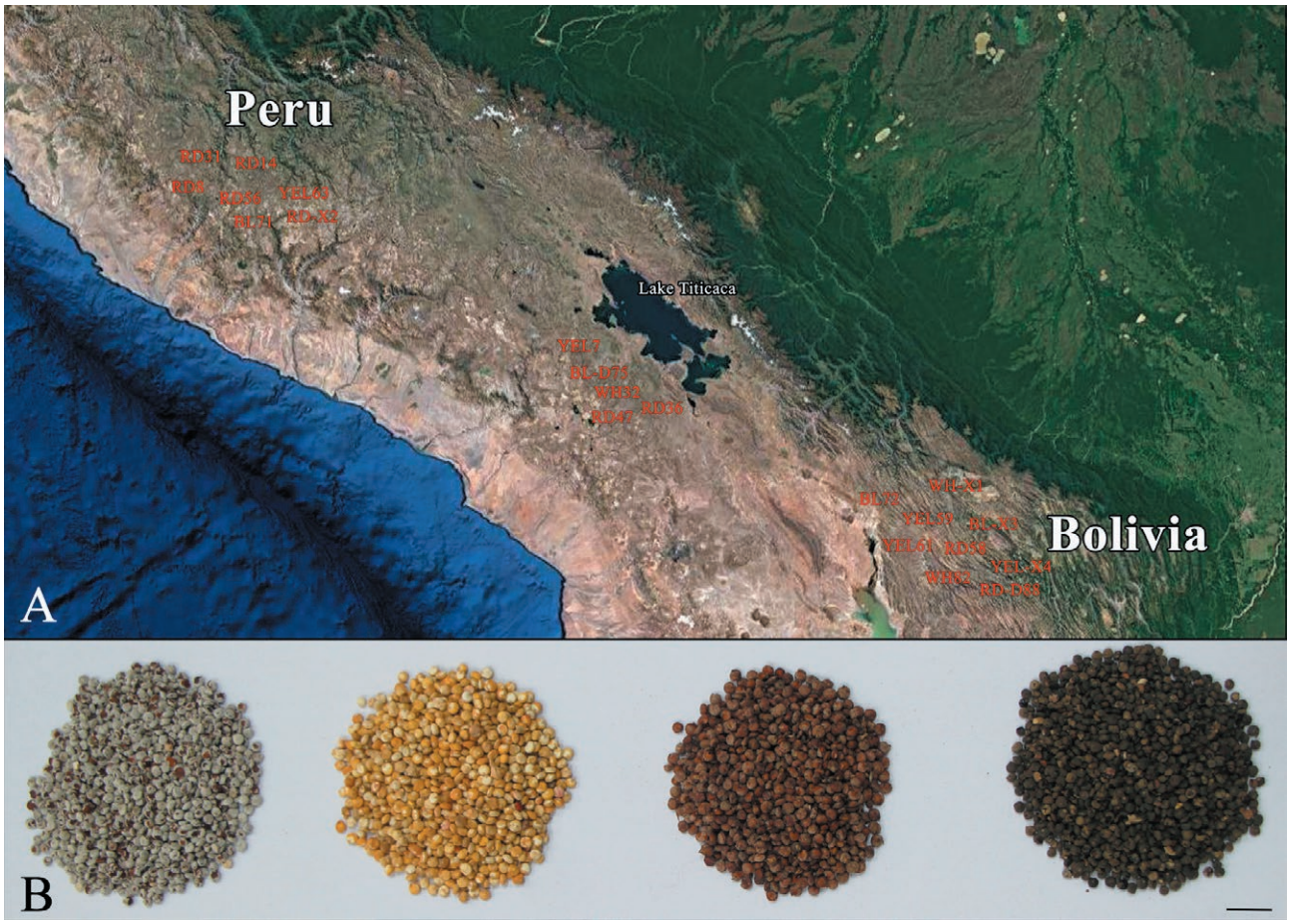

Figure 1. (A) Map showing the collection area of the studied cultivars (B) Photographs of seeds showing morphological variation (bar=1 cm).

metaphase plates were photographed with a mounted digital camera using the software ScopePhoto (ver. 3.0.12.444) (ScopeTek, Hangzhou, China).

Chromosome numbers were counted from at least 50 metaphase plates prepared from 50 different seedlings for each cultivar of quinoa to ensure the uniformity of somatic chromosome numbers in all the plants. Among them, at least 30 best metaphase plates were selected for karyotype analyses with a minimum of 10 seedlings chosen per cultivar.

Lengths of chromosome arms were measured from at least 5 best metaphase plates for each cultivar using KaryoType v2 software (Altınordu *et al.* 2016). The lengths of chromosomes and respective centromeric indices of each metaphase plate, obtained from this data, were used to describe the chromosome morphology following the nomenclatural system of Levan *et al.* (1964), and, to prepare their idiograms with OriginPro 2018 software (OriginLab Corporation, USA). Asymmetry of karyotypes of each cultivar were calculated using Stebbin's classification (1971) and AI (karyotype asymmetry index), intra-chromosomal asymmetry indices using A1 (intra-chromosomal asymmetry index), CV_{CI} (coefficient of variation of centromeric index), M_{CA} (mean centromeric asymmetry), Syi (symmetric index), AsK% (Karyotype asymmetry index percentage) and AR (arm ratio), and inter-chromosomal asymmetry using A2 (inter-chromosomal asymmetry index), CV_{CL} (coefficient of variation of chromosome length) (for details see Bhadra and Bandyopadhyay 2015). Pearson correlation among these intra- and inter-chromosomal asymmetry parameters was calculated in SPSS (version 21) software (München, Germany).

RESULTS

The chromosomes of studied cultivars of *Chenopodium quinoa* were small, though clearly visible with conspicuous centromeric regions.

Table 1. Chromosome numbers, karyotype formulae, morphometric parameters and values of asymmetry indices studies in 21 *Chenopodium quinoa* varieties.

Local variety name	2n	Karyotype formula	Short arm range (average) in μm	Long arm range (average) in μm	Total length range (average) in μm
BL71	36	2M+22m+8Sm+2St+2m:Sm	0.42–1.77 (1.03±0.25)	0.80–2.64 (1.49±0.34)	1.35–4.24 (2.56±0.50)
BL72	36	8M+14m+10m+2St+2m:Sm	0.42–1.92 (1.04±0.31)	0.50–3.02 (1.52±0.39)	0.92–4.19 (2.59±0.59)
BL–D75	36	18m+14Sm+2St+2M:Sm	0.39–2.15 (1.04±0.26)	0.46–2.83 (1.55±0.40)	0.85–4.51 (2.62±0.57)
BL–X3	36	6M+8m+16Sm+4St+2m:Sm	0.59–2.19 (1.21±0.28)	1.04–3.45 (1.91±0.36)	1.88–4.61 (3.16±0.42)
RD14	36	6M+14m+12Sm+2St+2m:Sm	0.52–1.79 (1.10±0.25)	0.65–2.88 (1.69±0.44)	1.17–3.96 (2.82±0.50)
RD31	36	4M+18m+10Sm+2St+2M:Sm	0.44–2.95 (1.13±0.33)	0.55–3.58 (1.71±0.42)	0.99–6.53 (2.88±0.66)
RD36	36	4M+26m+4Sm+2m:Sm	0.40–1.34 (0.95±0.18)	0.57–2.12 (1.30±0.23)	0.97–3.95 (2.28±0.38)
RD47	36	4M+20m+8Sm+2St+2m:Sm	0.40–1.83 (1.16±0.26)	0.46–2.82 (1.59±0.30)	0.86–4.41 (2.78±0.49)
RD56	36	8M+20m+6Sm+2M:Sm	0.37–1.48 (0.99±0.24)	0.49–2.09 (1.41±0.29)	0.86–3.85 (2.44±0.48)
RD58	36	8M+16m+8Sm+2St+2M:Sm	0.37–2.00 (1.13±0.37)	0.52–3.00 (1.60±0.44)	0.89–4.82 (2.76± 0.70)
RD8	36	2M+16m+12Sm+4St+2m:Sm	0.33–1.93 (1.10±0.30)	0.60–3.66 (1.67±0.44)	0.93–5.45 (2.81±0.64)
RD–D88	36	6M+16m+10Sm+2St+2m:Sm	0.42–1.85 (1.10±0.32)	0.50–2.96 (1.61± 0.35)	0.92–4.44 (2.75±0.58)
RD–X2	36	2M+12m+18Sm+2St+2m:Sm	0.25–1.64 (1.00±0.24)	0.38–2.38 (1.52±0.30)	0.63–3.94 (2.55±0.47)
WH32	36	6M+20m+6Sm+2St+2m:Sm	0.68–2.57 (1.18±0.32)	0.54–2.87 (1.77±0.40)	1.03–5.44 (2.99±0.58)
WH82	36	8M+22m+4Sm+2m:Sm	0.48–1.81 (1.10±0.26)	0.58–2.52 (1.49±0.32)	1.06–4.18 (2.62±0.54)
WH–X1	36	2M+18m+12Sm+2St+2m:Sm	0.35–1.42 (0.85±0.20)	0.38–2.42 (1.26±0.26)	0.73–3.20 (2.14±0.40)
YEL59	36	8M+18m+6Sm+2St+2M:Sm	0.49–1.97 (1.16±0.28)	0.73–2.83 (1.63±0.36)	1.22–4.34 (2.82±0.54)
YEL61	36	4M+16m+12Sm+2St+2m:Sm	0.42–1.66 (0.97±0.26)	0.47–2.91 (1.54±0.35)	0.92–3.98 (2.55±0.50)
YEL63	36	6M+22m+4Sm+2St+2m:Sm	0.47–1.75 (1.06±0.29)	0.59–2.81 (1.59±0.42)	1.06–4.36 (2.69±0.59)
YEL7	36	2M+22m+10Sm+2m:Sm	0.44–1.82 (1.16±0.26)	0.53–2.79 (1.70±0.36)	0.97–5.02 (2.92±0.54)
YEL–X4	36	4M+24m+6Sm+2m:Sm	0.53–1.68 (1.07±0.24)	0.77–2.61 (1.63±0.31)	1.35–4.50 (2.74±0.42)

Local variety name	Centromeric index range (average) (i)	Karyotype asymmetry class	Arm ratio range (average) (AR)	Value of relative chromatin (VRC)	Total form percentage (TF%)	Relative length percentage range (RL)
BL71	18.79–49.76 (41.17±6.83)	2B	0.70–4.32 (1.51±0.06)	5.11±0.61	41.78±0.96	1.56–4.56
BL72	16.35–49.76 (40.52±7.72)	2B	0.69–5.12 (1.57±0.11)	5.19±0.64	41.35±1.06	0.86–4.06
BL–D75	19.43–49.70 (40.52±6.37)	2B	0.57–4.14 (1.54±0.13)	5.24±0.99	40.80±2.13	0.93–4.46
BL–X3	21.69–49.82 (39.40±7.63)	2B	0.41–3.61 (1.65±0.22)	6.32±0.88	39.76±3.18	1.91–3.64
RD14	14.79–50 (40.01±8.03)	2B	0.54–5.76 (1.62±0.11)	5.65±0.13	40.21±0.36	1.63–3.95
RD31	22.41–49.78 (40.13±6.44)	2B	0.61–3.46 (1.59±0.06)	5.76±1.52	40.33±0.93	1.20–6.74
RD36	31.22–49.76 (42.48±4.17)	2B	0.60–2.20 (1.38±0.02)	4.56±0.47	42.98±0.27	1.26–4.30
RD47	24.31–49.84 (42.36±5.77)	2B	0.77–3.11 (1.43±0.13)	5.56±0.56	42.77±1.88	0.96–4.02
RD56	25.73–49.84 (41.84±5.65)	2B	0.60–2.88 (1.46±0.09)	4.87±0.22	42.26±1.37	0.95–4.29
RD58	15.56–49.81 (41.44±7.46)	2B	0.68–5.42 (1.52±0.10)	5.53±0.89	42.08±0.96	0.89–4.57
RD8	18.16–49.32 (40.02±6.75)	2B	0.55–4.51 (1.59±0.21)	5.63±0.46	40.52±2.49	0.97–4.92
RD–D88	21.32–49.82 (40.35±7.40)	2B	0.39–3.69 (1.57±0.12)	5.49±0.81	41.34±1.44	1.10–5.26
RD–X2	20.92–50 (39.99±6.66)	2B	0.54–3.78 (1.59±0.28)	5.10±0.06	40.48±4.02	0.68–4.24
WH32	19.82–49.64 (40.52±7.08)	2B	0.53–4.04 (1.58±0.09)	5.98±0.45	40.63±1.43	0.88–4.65
WH82	25.00–49.72 (42.38±5.24)	2B	0.70–3.00 (1.38±0.04)	5.25±0.72	43.23±0.71	1.06–4.07
WH–X1	24.37–50 (40.97±5.53)	2B	0.44–3.10 (1.51± 0.20)	4.29±0.37	41.30±2.96	1.01–3.88
YEL59	22.09–49.77 (41.92±6.15)	2B	0.76–3.53 (1.46±0.10)	5.64±0.88	42.38±1.52	1.29–3.99
YEL61	18.03–49.69 (39.05±7.23)	2B	0.34–4.55 (1.66±0.16)	5.09±0.59	39.69±1.53	1.04–4.49
YEL63	17.07–49.83 (40.57±7.38)	2B	0.41–4.86 (1.56±0.10)	5.39±0.53	40.99±2.15	1.04–4.26
YEL7	25.69–49.60 (40.97±6.96)	2B	0.67–3.28 (1.51±0.05)	5.84±0.46	41.69±0.48	0.85–4.41
YEL–X4	18.03–50 (39.87±6.91)	2B	0.35–4.55 (1.62±0.41)	5.47±0.28	40.49±4.74	1.35–4.51

Local variety name	Difference of relative length (DRL)	Intra–chromosomal asymmetry index (A1)	Inter–chromosomal asymmetry index (A2)	Coefficient of variation of chromosome length (CV_{CL})	Coefficient of variation of centromeric index (CV_{CI})
BL71	2.52±0.54	0.29±0.02	0.19±0.03	19.46±2.92	16.61±1.14
BL72	2.72±0.30	0.29±0.03	0.22±0.03	22.48±2.72	18.61±1.68
BL–D75	2.92±0.15	0.31±0.06	0.22±0.04	22.35±3.63	15.79±1.83
BL–X3	1.49±0.30	0.33±0.07	0.14±0.03	13.71±3.18	19.58±4.55
RD14	2.18±0.06	0.31±0.01	0.18±0.03	17.65±2.80	20.08±3.43
RD31	4.65±0.95	0.33±0.02	0.23±0.05	23.28±4.90	16.04±2.22
RD36	2.54±0.30	0.26±0.01	0.17±0.01	16.75±1.13	9.80±0.38
RD47	2.48±0.18	0.26±0.05	0.18±0.01	17.78±0.12	13.66±2.14
RD56	2.62±0.38	0.28±0.03	0.20±0.01	19.76±0.73	13.56±3.10
RD58	3.07±0.14	0.27±0.01	0.25±0.02	25.17±2.28	18.04±4.42
RD8	3.06±0.34	0.32±0.06	0.23±0.02	22.78±2.36	17.10±4.25
RD–D88	3.11±1.01	0.30±0.04	0.21±0.05	21.48±5.02	18.46±3.91
RD–X2	2.90±0.59	0.32±0.10	0.18±0.03	18.42±2.72	16.81±2.54
WH32	2.73±0.74	0.31±0.04	0.20±0.03	19.47±2.67	17.54±2.46
WH82	2.56±0.27	0.25±0.02	0.20±0.03	20.35±3.37	12.35±0.94
WH–X1	2.29±0.46	0.30±0.08	0.19±0.02	18.78±1.74	13.68±3.97
YEL59	2.34±0.27	0.27±0.03	0.19±0.02	19.44±2.22	14.69±3.76
YEL61	2.75±0.95	0.34±0.03	0.20±0.05	20.14±4.61	18.63±5.77
YEL63	2.53±0.70	0.30±0.05	0.22±0.04	22.05±4.30	18.24±2.49
YEL7	2.89±0.60	0.29±0.02	0.18±0.02	18.32±2.18	14.65±0.44
YEL–X4	2.26±0.80	0.32±0.12	0.15±0.03	15.52±3.59	17.93±7.93

Local variety name	Karyotype asymmetry index (AI)	Mean centromeric asymmetry (M_{CA})	Symmetric index (Syi)	Karyotype asymmetry index percentage (AsK%)
BL71	3.21±0.24	16.80±1.39	144.12±5.02	58.22±0.96
BL72	4.20±0.83	17.47±2.05	146.47±6.20	58.65±1.06
BL–D75	3.51±0.51	18.30±3.74	150.64±14.17	59.20±2.13
BL–X3	2.59±0.23	20.43±5.43	157.21±20.92	60.24±3.18
RD14	3.48±0.18	19.08±0.69	154.02±2.09	59.79±0.36
RD31	3.66±0.27	19.46±1.48	153.07±5.90	59.67±0.93
RD36	1.64±0.05	14.55±0.73	136.77±2.15	57.02±0.27
RD47	2.43±0.39	15.04±3.37	138.12±10.76	57.23±1.88
RD56	2.69±0.70	16.18±2.37	142.12±7.60	57.73±1.37
RD58	4.61±1.47	16.34±1.52	141.87±5.68	57.92±0.96
RD8	3.91±1.15	19.02±4.76	152.55±17.95	59.48±2.49
RD–D88	3.97±1.37	18.32±3.14	147.19±9.70	58.66±1.44
RD–X2	3.14±0.94	19.26±7.26	153.63±27.35	59.52±4.02
WH32	3.41±0.60	18.70±2.72	150.94±9.46	59.37±1.43
WH82	2.53±0.57	14.24±1.45	135.69±3.16	56.77±0.71
WH–X1	2.53±0.54	17.70±5.75	147.95±18.65	58.70±2.96
YEL59	2.81±0.45	15.52±2.37	140.52±9.14	57.62±1.52
YEL61	3.57±0.18	20.85±2.73	158.16±9.74	60.31±1.53
YEL63	4.09±1.36	17.98±3.48	149.65±12.98	59.01±2.15
YEL7	2.68±0.27	17.03±1.24	145.88±4.32	58.31±0.48
YEL–X4	2.66±0.84	19.28±8.95	154.89±32.99	59.51±4.74

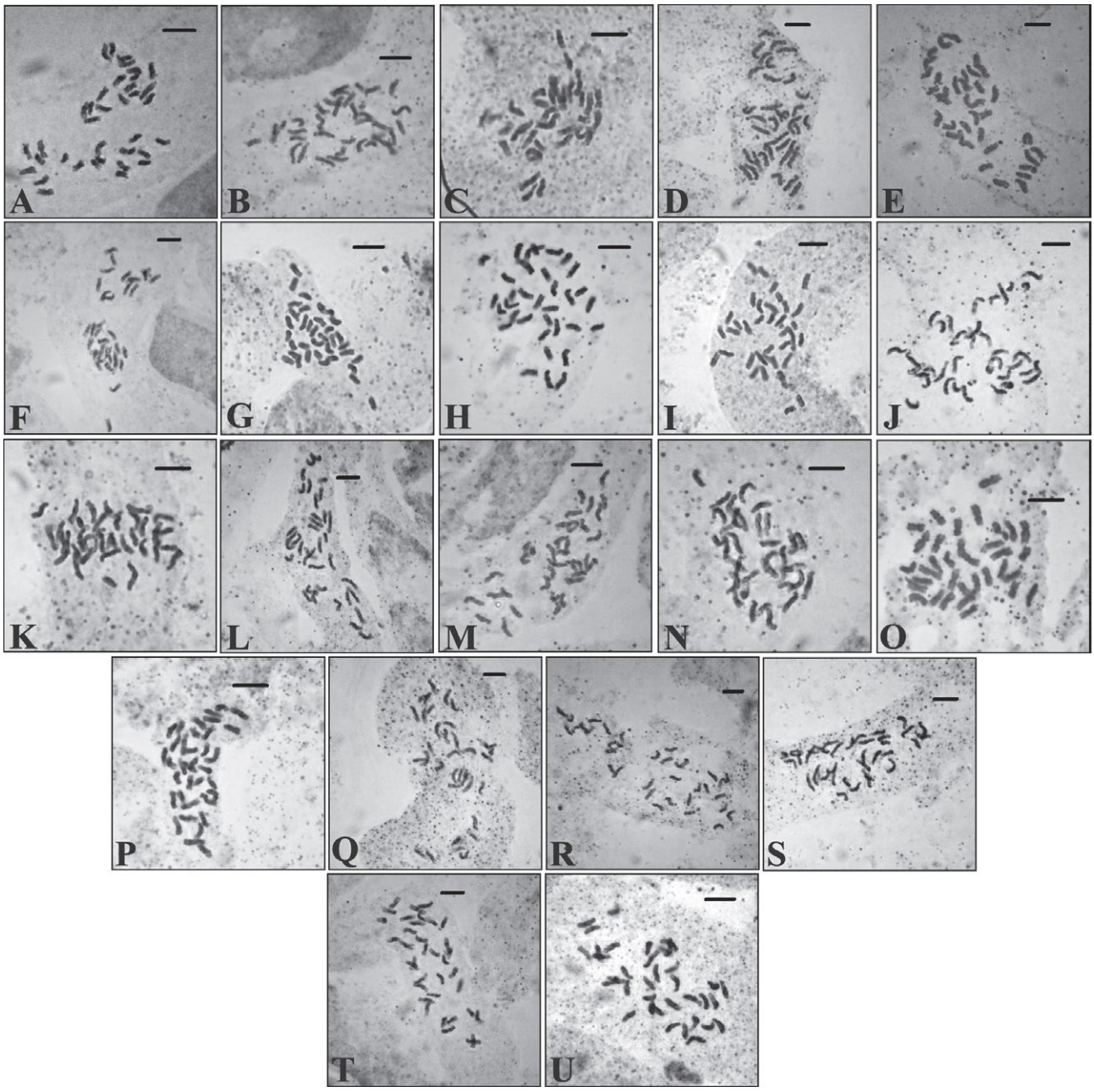

Figure 2. Somatic metaphase chromosomes of *Chenopodium quinoa* (2n=36): (A) BL71 (B) BL72 (C) BL–D75 (D) BL–X3 (E) RD14 (F) RD31 (G) RD36 (H) RD47 (I) RD56 (J) RD58 (K) RD8 (L) RD–D88 (M) RD–X2 (N) WH32 (O) WH82 (P) WH–X1 (Q) YEL59 (R) YEL61 (S) YEL63 (T) YEL71 (U) YEL–X4 (bar= 5 μm).

Somatic cells of all the cultivars exhibited a uniform chromosome number of 2n= 36 (Table 1, Figure 2). Lengths of chromosomes in all the cultivars varied from 0.63 μm to 6.53 μm, with the highest average chromosome length being present in the cultivar BL–X3 and the lowest in WH–X1 (Figure 3 A–F). Short arms were 0.25 μm to 2.95 μm in length, while long arms showed a variation of 0.38 μm to 3.66 μm. Centromeric indices ranged from 50% to 14.79% resulting in primary constrictions varying from median to sub-terminal types, though one cultivar did not possess any chromosome with median constriction and chromosomes of five cultivars did not exhibit sub-terminal constrictions. Number of chromosomes with each type of primary constriction also var-

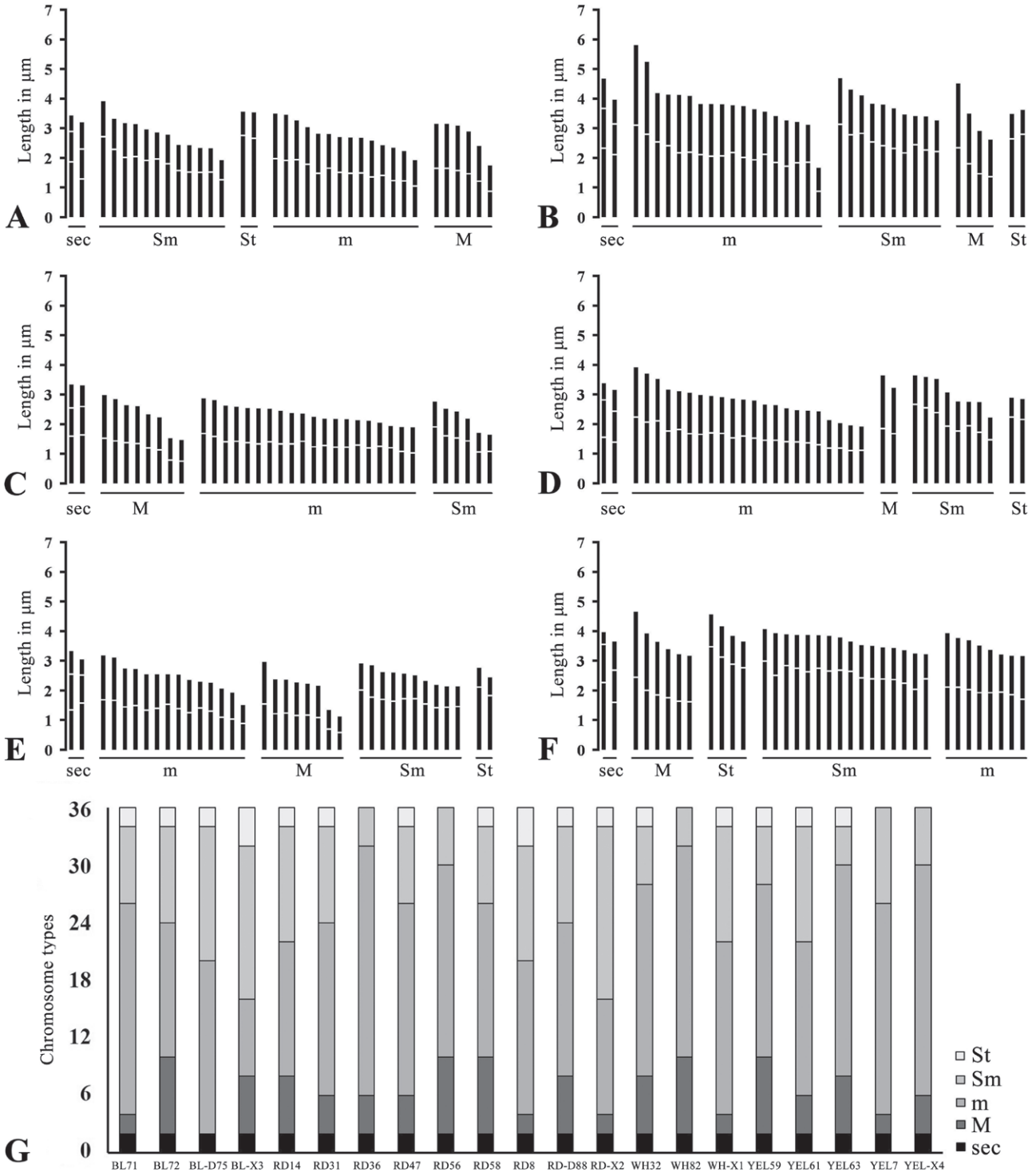

Figure 3. Idiograms of some cultivars of *Chenopodium quinoa*: (A) RD14 (B) RD31 (C) RD56 (D) BL71 (E) BL72 (F) BL–X3 (G) Comparative graphical representation of distribution of constriction types in the cultivars of *Chenopodium quinoa* (M= median constriction, m= nearly median constriction, Sm= sub–median constriction, St= sub–terminal constriction, sec= secondary constriction).

ied among the cultivars. All of the cultivars revealed 2 chromosomes possessing secondary constrictions, with one constriction in median or nearly median region and the other in sub–median region (Figure 3 G).

The inter- and intra-chromosomal asymmetry indices were calculated on the basis of chromosome lengths and centromeric indices. Stebbins classification (1971) placed the karyotype of quinoa in 2B category while AI ranged from 1.64 in RD36 to 4.61 in RD58. The complementary indices A1 and A2 ranged from 0.25 to 0.34, and, 0.15 to 0.25 respectively. Values of CV_{CL} and CV_{CI} varied from 15.52–25.17, and, 9.80–20.08 respectively, while that of M_{CA} was 14.24–20.85. Values of average AR ranged from 1.38 to 1.66, with the lowest recorded in YEL61 and highest in RD14. The values of Syi and Ask% ranged from 135.69 to 158.16, and, 56.77% to 60.31% respectively.

DISCUSSION

Domestication of plants has been impacted by polyploidy since the beginning of agriculture, with approximately 40–70% of cultivated plants exhibiting polyploidy (Hilu 1993, Sattler *et al.* 2016). Polyploids, especially allopolyploids show hybrid vigour with increased growth rates and higher productivity that favours their artificial selection over their diploid progenitors (Renny–Byfield and Wendel 2014). Considering Goldblatt's (1980) assumption of polyploid determination, quinoa with the basic chromosome number of x= 9, is a tetraploid with 2n= 36 somatic chromosomes, a condition similar to the closely related species *C. hircinum* and *C. berlandieri* (Fuentes–Bazan *et al.* 2012; Mandák *et al.* 2016; Jarvis *et al.* 2017). Studies have endorsed its allotetraploid nature, hypothesizing possible hybridization between a North American and a Eurasian diploid species, whose identities are yet unknown (Ward 2000; Jarvis *et al.* 2017). However, polyploid establishment and propagation is often hindered by irregular meiotic segregation of chromosomes that lead to chromosomal abnormality and reproductive sterility (Renny–Byfield and Wendel 2014). On the contrary, stability in the number of somatic chromosomes of this species, especially in those cutivars that were reported from Bolivia, Chile and Peru (Cárdenas and Hawkes 1948; Gandarillas and Luizaga 1967; Kolano *et al.* 2012), and the apparent absence of evidences of mixoploidy in the highland varieties investigated in the present study, both failed to give credence to sporadic reports of mixoploidy (Kawatani and Ohno 1950; Gandarillas 1979; Wang *et al.* 1993). This could in effect perhaps lead to the high fertility of quinoa (Ward 2000) that, along with its self–pollinating nature, has facilitated its extensive propagation, especially in new regions.

Karyomorphological trait variations resulting from structural changes of chromosomes, primarily as a result of rearrangement of chromosomal parts including translocation, inversion and/or deletion, is an important character for understanding relationship among closely related taxa (Peruzzi and Altinordu 2014). However, no published record of structural details of somatic chromosomes of quinoa was available until the beginning of this century. Present study recorded small somatic chromosomes with higher length variations, between 0.63–6.53 µm, and the longest chromosome was almost double the length of previously recorded longest chromosome which was 3.30 µm (Kolano *et al.* 2001; Palomino *et al.* 2008; Yangquanwei *et al.* 2013). This difference can be attributed to (a) the variable cytological techniques used in these studies that affect the degree of chromosomal condensation (Palomino *et al.* 2008), and, (b) the use of only 1–2 varieties of quinoa in the previous studies that exempted any scope of understanding existing cytological variations in the species. The latter reason stated here might also be true for the limited variations observed in the position of primary constrictions in the past studies. Chromosomes with only nearly median constrictions were observed by Palomino *et al.* (2008) and Bhargava *et al.* (2006), with only a single variety revealing 2 chromosomes with sub-median constrictions being reported by Bhargava *et al.* (2006). This was in contrast with the presence of sub–terminal constrictions in about 75% of the varieties of quinoa investigated in the present study revealing higher degree of intra–chromosomal variations (Table 1, Figure 3G). The number of chromosomes possessing secondary constrictions in each complement of all the cultivars in the present study, corroborated observations of Bhargava et al (2006), though contrasting observations of 2 pairs of secondary constrictions were reported by Palomino et al (2008). However, some other studies have reported absence of chromosomes with secondary constrictions (Cárdenas and Hawkes 1948; Gandarillas and Luizaga 1967; Giusti 1970; Gandarillas 1979). Chromosomes possessing secondary constriction had median or nearly median primary constriction and secondary constrictions in sub–median position. Gross chromosome morphology obtained in the present study, although showed significant variations in both chromosome length and constrictions, did not reveal any characteristic pattern that can be related to the geography or seed morphology of the studied cultivars.

Study of chromosomal characteristics forms the basis of cytotaxonomy that can be used to understand similarity or divergence among related species, and is based

on asymmetry indices calculated with the help of chromosome measurements (Levitzky 1931; Stebbins 1971). Since asymmetry is an expression of chromosomal morphology, it is important to compare karyotypes on appropriate statistical grounds and choose correct statistical parameters (Peruzzi and Altinordu 2014). However, there is a lack of general consensus on the specific karyotype asymmetry parameter to be utilized which necessitates use of multiple available parameters to conclude about the asymmetry status of a taxa and its comparison with relatives. Inter-chromosomal asymmetry increases with increasing difference between the lengths of smallest and largest chromosomes of a complement, and, are assessed by calculating A2 and CV_{CL}. Intra-chromosomal asymmetry increases with shift in centromeric position from median to terminal constriction in a complement. This is assessed by calculating the values of TF%, Syi, AsK%, A1, M_{CA} and CV_{CI} (Paszko 2006; Peruzzi and Eroğlu 2013).

In the present investigation, the different cultivars of quinoa showed significantly higher asymmetry than former studies as was evident by the karyotype asymmetry indices (Table 1). Stebbins asymmetry class, where all the cultivars of the present study were classified as 2B karyotype, endorsed more asymmetric karyotype than 1A and 1B obtained from 7 cultivars by Bhargava *et al.* (2006). AI, with an average value of 3.21±0.62 also indicate the same. However, calculation of these 2 indices are based on a combination of inter- and intra-chromosomal asymmetry, which has been suggested against in recent studies (Peruzzi and Eroglu 2013). TF% that decreased with increasing asymmetry ranged from 39.69–43.23% with an average of 41.29±0.86%, lower than previously reported values of 43.8–47.4% (Bhargava *et al.* 2006; Palomino *et al.* 2008) affirming higher asymmetry revealed in the present study. Similarly, evaluation of AR that ranged from 0.34 to 5.76 in the present study, but showed much less variation (1.00–1.86) revealing lesser asymmetry in the previous studies also imply presence of higher variation among the cultivars assessed in the present study (Bhargava *et al.* 2006; Palomino *et al.* 2008). Other asymmetry indices examined in the present study also supported the above contention as was evident by the calculated Pearson correlation. Inter–chromosomal and intra–chromosomal asymmetry indices revealed strong correlation within themselves (Table 2). TF% was found to be negatively correlated with other intra–chromosomal indices of A1 (r= −0.978), CV_{CI} (r= −0.770), M_{CA} (r= −0.991), Syi (r= −0.995) and Ask% (r= −1.000) justifying increasing intra-chromosomal asymmetry with decreasing value of TF%. A2 was positively correlated with CV_{CL} (r= 0.992) indicating increasing value with increasing differences in chromosome size. However, inter– chromosomal asymmetry indices were weakly correlated with intra–chromosomal indices, justifying the suggestion of not including both type of indices in a single analysis (Peruzzi and Eroglu 2013).

Both inter- and intra-chromosomal asymmetry are necessary to reveal correlation among related taxa. This is best represented by using bi-dimensional scatter plots that include one inter-chromosomal and one intra-chromosomal parameter in each axis of the plot (Peruzzi and Eroğlu 2013). Restricted distribution of cultivars in the scatter plots generated in the present study comparing the values of A1 versus A2, CV_{CI} versus CV_{CL}, and, M_{CA} versus CV_{CL} indicated high similarity among the cultivars with respect to chromosomal asymmetry (Figure 4). The plot indicated that, although the study revealed consistent differences within the karyotype of each cultivar, the characteristic distinctions were inadequate for cultivar identification, and not related to their geographical origin and seed morphology.

Overall, high inter- and intra-chromosomal variability in the quinoa cultivars examined here conclusively exhibited that much variation exists in this species that is yet to be explored exhaustively. During early domestication events, conspicuous divergence of domesticated

Table 2. Values of Pearson correlation analysis for intra- and inter-chromosomal asymmetry indices

	A1	A2	CV_{CL}	CV_{CI}	M_{CA}	TF	Syi	AsK
A1	1							
A2	−0.071	1						
CV_{CL}	−0.073	0.992*	1					
CV_{CI}	0.667*	0.110	0.118	1				
M_{CA}	0.985*	−0.067	−0.070	0.765*	1			
TF	−0.978*	0.070	0.079	−0.770*	−0.991*	1		
Syi	0.981*	−0.121	−0.125	0.745*	0.990*	−0.995*	1	
AsK	0.977*	−0.070	−0.079	0.770*	0.991*	−1.000*	0.994*	1

*Correlation is significant at 0.01 level.

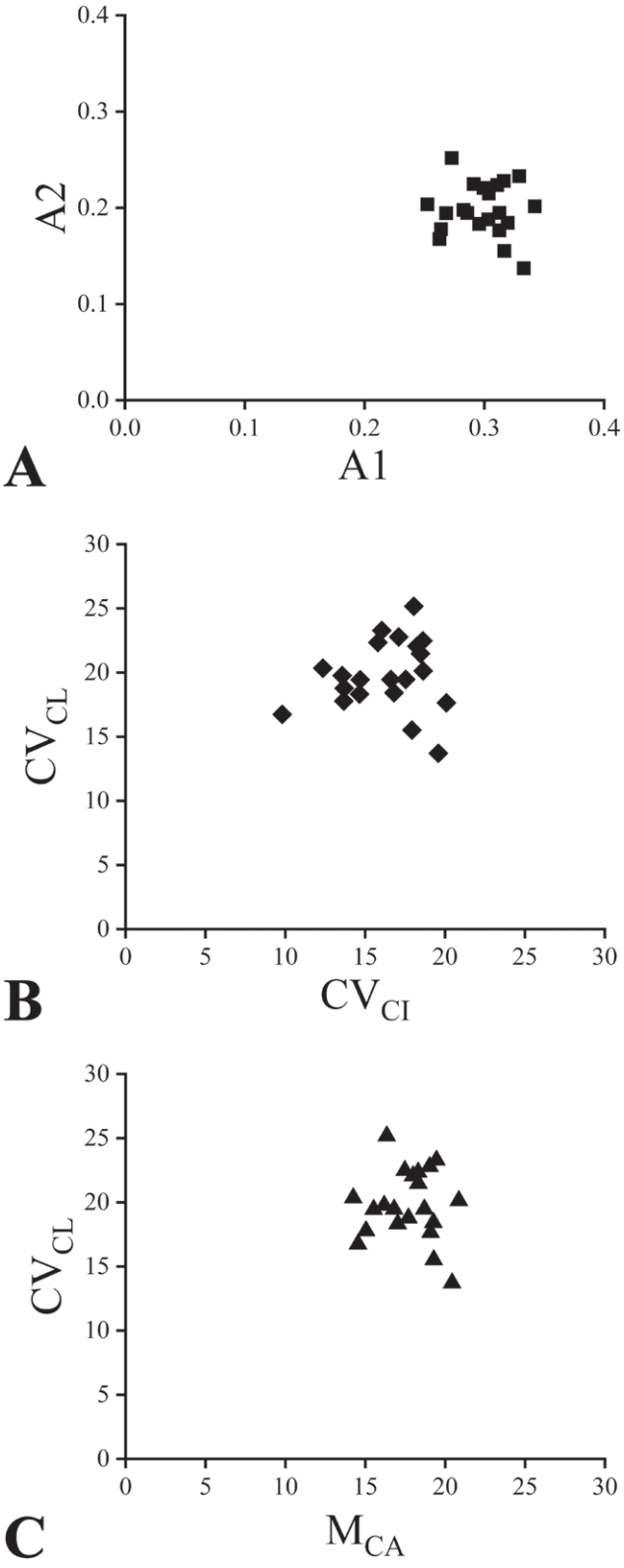

Figure 4. Scatter diagrams for the *Chenopodium quinoa* cultivars of (A) A1 against A2 (B) CV_{CI} against CV_{CL} (C) M_{CA} against CV_{CL}.

species from their wild relatives resulted from artificial selection and controlled reproduction, especially cross–fertilization, by human intervention, that ended in genetic bottleneck, thereby reducing genetic diversity of cultivated plants with respect to their wild counterparts (Tanksley and McCouch 1997; Cornille *et al.* 2014). Along with this, early farmers selected few individuals with more desirable traits for next generation cultivation with little effort of conserving the unselected materials that were being pushed to oblivion, thereby causing severe loss of genetic diversity and limiting the gene pool of present day domesticated plants (Pan *et al.* 2016). In recent years, cultivation of local varieties of quinoa has been largely neglected because of increased pressure on local farmers for production of certified high–yielding quinoa varieties in international quinoa market (Salazar *et al.* 2019). The inter–varietal similarity and stability of chromosomal characteristics observed in our studied quinoa cultivars might be an indication of the bottleneck experienced by the plants due to their long domestication history of over 7,000 years (Jarvis *et al.* 2017; Salazar *et al.* 2019), that had allowed enough time to impart homogeneity and stability in their chromosomal structures. Loss of diversity in local quinoa gene pool in this way will pose serious threat in future breeding programs for quality improvement that necessitates an imperative exploration of chromosomal variability (Jarvis *et al.* 2017; Salazar *et al.* 2019). This entails an imperative investigation of inter–varietal chromosomal diversity in quinoa that would include chromosomal fluorescent staining techniques, like FISH, incorporating a large number of cultivars encompassing varieties growing on the distinct eco–geographical regions of quinoa cultivation, primarily from the highland and the coastal lowland ecotypes. It will provide a comprehensive basis for evaluation, selection, conservation and maintenance of existing germplasm, thereby assisting in future breeding programs.

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