Seed-Set Efficiency and Seed Quality in Ten *Calendula officinalis* L. Cultivars: Implications for Seed-Production Selection in Uzbekistan

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Abstract

Choosing cultivars for seed multiplication requires knowing not only how many seeds a crop produces but how efficiently its reproductive potential is converted into seed. We quantified potential seed productivity (PSP, fertile ray florets per capitulum), realized seed productivity (RSP, fully developed achenes per capitulum) and the seed productivity coefficient (SPC = RSP/PSP × 100) in ten *Calendula officinalis* L. cultivars grown in single, unreplicated plots under irrigation in the Tashkent Region, Uzbekistan (2023-2024). Florets and achenes were counted in 100 mature capitula per cultivar. Capitulum PSP ranged from 35 to 47, RSP from 22 to 36 and SPC from 62.9 to 76.6%. The number of florets that failed to set seed was nearly constant across cultivars (11-13 per capitulum), and a constant-loss model described the PSP-RSP relationship far better than a proportional-loss model (ΔAIC=16.2). Consequently, cultivars with larger capitula realized a greater share of their potential. Mature achenes per plant varied less among cultivars (1,762-2,286; CV 7.1%) than achenes per capitulum (CV 15.3%), suggesting partial compensation between capitulum size and number. Germination of the harvested seed (54-96%) increased with capitulum SPC (Spearman ρ = 0.77; 95% bootstrap CI 0.21-1.00). In a principal component analysis, the first component (80.5% of variance) combined all reproductive and seed-quality traits and separated ‘Orange Porcupine’, ‘Orange King’ and ‘Radio’ from the remaining cultivars. Because plots were not replicated, cultivar differences are descriptive; the three leading cultivars are proposed for replicated, multi-year seed-production trials.

Keywords: *Calendula officinalis*; seed production; potential seed productivity; seed set; ovule loss; seed quality; cultivar screening

1. Introduction

Pot marigold (*Calendula officinalis* L., Asteraceae) is grown for medicinal raw material, ornamental use and seed oil rich in calendic acid, and all of these uses depend on a reliable supply of viable, true-to-type seed [1]. Seed yield of calendula depends on the number of flower heads, the proportion of achene morphs, harvest date and weather [2, 5], and its response to plant density and sowing rate has been studied in Europe [1, 3]. Yield alone, however, says little about how seed output is formed. It results from the number of seed-forming units initiated and from the proportion of them that develop into mature seeds.

Reproductive-biology methods separate these two components. Potential seed productivity (PSP) is the number of ovules, or ovule-bearing florets, that could in principle form seeds; realized seed productivity (RSP) is the number of fully

developed seeds; and their ratio expresses how completely the potential is realized [6, 7]. Seed-to-ovule ratios below unity are the rule in flowering plants and arise from pollen limitation, resource limitation, abortion and positional effects within inflorescences [12-14]. Whether ovule loss is proportional to the number of ovules, or roughly constant in absolute terms, matters for selection: under proportional loss, larger inflorescences gain seeds in proportion to their size, whereas under constant loss they also gain efficiency.

The calendula capitulum is heterogamous. Ray florets are female and fertile, each with a single ovule, whereas disc florets are functionally male; the achenes are arranged in several rows and are strongly heteromorphic [8]. Cultivars differ in the number of ray-floret rows and in ray-floret form, which changes the number of potential seed-forming units per capitulum.

Quantitative data on potential and realized seed productivity of calendula remain scarce. In the Perm region of Russia, a single cultivar formed about 448 seeds from 1,067 ovules per plant [4], and cultivars were compared for seed propagation and germination in Western Siberia [11]. We are not aware of studies that partition capitulum-level reproductive capacity and its realization across several cultivars under the irrigated, sharply continental conditions of Central Asia. The objectives were to (i) quantify PSP, RSP and SPC at the capitulum level in ten cultivars; (ii) test whether floret loss is constant or proportional to capitulum size; (iii) examine plant-level seed output and its relation to capitulum traits; (iv) relate these traits to the quality of the harvested seed; and (v) identify cultivars that merit replicated seed-production trials.

2. Materials and methods

2.1. Study site, soil and weather

The study was carried out in 2023-2024 at the experimental field of the Institute of Plant Genetic Resources, Qibray district, Tashkent Region, Uzbekistan (41.46° N, 69.38° E; about 486 m a.s.l.; groundwater table at 5-7 m). The soil is a long-irrigated typical serozem of silty medium-loam texture. In the 0-20 and 20-50 cm layers, humus content was 1.58 and 1.40%, total nitrogen 0.016 and 0.013% and exchangeable K_2 O 170 and 138 mg kg^{-1}; mobile P_2 O_5 in the 0-20 cm layer was 21.2 mg kg^{-1} and pH was about 7.7. The previous crop was garlic in both seasons. The climate is sharply continental, with a hot, dry summer. Spring rainfall differed strongly between seasons (79.8 mm from March to June in 2023 and 199.9 mm in 2024), whereas summer months were almost rainless in both years (Table 1).

Table 1. Monthly precipitation (mm) at the Akkavak meteorological station (Uzhydromet), March-September 2023-2024.

Year	Mar	Apr	May	Jun	Jul	Aug	Sep
2023	34.5	33.3	10.7	1.3	3.6	5.3	5.2
2024	96.5	29.9	70.3	3.2	8.3	0.6	4.0

2.2. Plant material

Ten cultivars were studied: ‘Radio’ (reference), ‘Bon Bon’, ‘Orange King’, ‘Золотая Принцесса’ (‘Golden Princessa’), ‘Indian Prince’, ‘Nova’, ‘Neon’, ‘Orange Porcupine’, ‘Geisha Girl’ and ‘Calexis Orange’. ‘Radio’ and ‘Orange Porcupine’ have quilled ray florets and ‘Geisha Girl’ has incurved, chrysanthemum-like capitula. The sowing seed had a purity of 90-95% and a germination of 84-95%.

2.3. Field design and management

Each cultivar was sown in a single plot of 35 m² (total area 350 m²) without field replication. Seeds were sown on 19 March at 70 × 30 cm (47,619 plants ha^{-1}) to a depth of 2-3 cm. Farmyard manure (30 t ha^{-1}), P_2O_5 (90-120 kg ha^{-1}) and KCl (60-90 kg ha^{-1}) were applied in autumn before ploughing to 25-27 cm; nitrogen (20-30 kg ha^{-1}) was applied at the 3-4-leaf stage and P and K (40-50 kg ha^{-1}) before flowering. Plots were irrigated every 7-10 days after emergence and every 5-7 days during flowering. Capitula with ripe seed were hand-picked in several passes, from late May to September.

2.4. Reproductive traits

Reproductive traits were determined following Vainagiy [6] and Levina [7]. For each cultivar, four samples of 25 fully mature, intact capitula (n = 100 capitula per cultivar) were taken from different parts of the plot, dissected and counted under an MBS-10 stereomicroscope. PSP was the number of fertile ray florets per capitulum, each carrying one ovule, and RSP the number of fully developed, mature achenes; shrivelled, empty, parthenocarpic and aborted structures were not counted. SPC (%) was calculated as RSP/PSP×100. Achene morphs were not counted separately.

Mature achenes per plant (plant RSP) were counted on 10 plants per cultivar taken from different parts of the plot. The number of seed-bearing capitula per plant was not counted independently; it is reported as an estimate obtained by dividing plant RSP by capitulum RSP and is used only descriptively.

2.5. Seed quality

Germination energy and germination of the harvested seed were determined on four replicates of 100 seeds incubated at 25 °C in Petri dishes in a TS-80M-2 thermostat according to GOST 12038-84 [9]. The mass of 1,000 seeds was determined according to GOST 12042-80 [10].

2.6. Data analysis

Because each cultivar occupied one unreplicated plot, cultivar differences cannot be tested against field error [15]; cultivar values are therefore reported as descriptive means, and no analysis of variance among cultivars is presented. To test the form of floret loss, two one-parameter models were fitted to the ten cultivar means by least squares: a constant-loss model, $RSP=PSP-c$, and a proportional-loss model, $RSP=k\cdot PSP$. They were compared by root-mean-square error and Akaike’s information criterion ($AIC=n\cdot\ln(SSE/n)+2p$); an unconstrained linear regression was fitted for reference. Associations among cultivar means were assessed with Pearson’s r and Spearman’s ρ, with 95% confidence intervals from 10,000 bootstrap resamples of cultivars; because SPC is calculated from PSP and RSP, correlations of SPC with either trait are partly arithmetic and are not interpreted as independent evidence.

Multivariate structure was summarized by principal component analysis (PCA) of seven standardized traits (capitulum PSP, RSP and SPC, plant RSP, germination energy, germination and 1,000-seed mass). Descriptive statistics followed Dospekhov [16]; models, bootstrap and PCA were computed in Python (NumPy, SciPy). All inferential results rest on ten cultivar means and are treated as exploratory.

3. Results

3.1. Capitulum-level PSP, RSP and SPC

Capitulum PSP ranged from 35 ray florets in ‘Golden Princessa’ to 47 in ‘Orange Porcupine’ (mean 40.9), and RSP from 22 to 36 mature achenes (mean 28.7; Table 2; Fig. 1a). ‘Orange Porcupine’, ‘Radio’ and ‘Orange King’ had the highest values, and ‘Golden Princessa’, ‘Geisha Girl’ and ‘Indian Prince’ the lowest. SPC ranged from 62.9% in ‘Golden Princessa’ to 76.6% in ‘Orange Porcupine’, with ‘Orange King’ (75.0%) and ‘Radio’ (73.3%) next.

Table 2. Capitulum-level potential (PSP) and realized (RSP) seed productivity, unrealized florets and seed productivity coefficient (SPC) of ten *C. officinalis* cultivars (Tashkent Region, 2023-2024).

Cultivar	PSP (florets)	RSP (achenes)	PSP − RSP	SPC (%)
Radio (reference)	45	33	12	73.3
Bon Bon	40	27	13	67.5
Orange King	44	33	11	75.0
Golden Princessa	35	22	13	62.9
Indian Prince	38	25	13	65.8
Nova	41	28	13	68.3
Neon	43	31	12	72.1
Orange Porcupine	47	36	11	76.6
Geisha Girl	37	25	12	67.6
Calexis Orange	39	27	12	69.2
Mean ± SD	40.9 ± 3.8	28.7 ± 4.4	12.2 ± 0.8	69.8 ± 4.3

Values are means of 100 capitula per cultivar from a single unreplicated plot. Mean ± SD is calculated among the ten cultivar means.

3.2. Floret loss is nearly constant

The number of ray florets that did not develop into mature achenes was similar in all cultivars, 11-13 per capitulum (mean 12.2; CV 6.5%), whereas PSP varied more (CV 9.3%). The constant-loss model fitted the cultivar means much better than the proportional-loss model (RMSE 0.75 vs 1.68 achenes; AIC −3.8 vs 12.4; Fig. 1b). The unconstrained regression had a slope of 1.14 (95% CI 1.02-1.26), well above the

proportional-loss expectation (0.71) and close to, though slightly above, the constant-loss expectation (1.0), indicating that the absolute loss, if anything, declined slightly in larger capitula. Consequently, cultivars with more ray florets lost a smaller proportion of them, and SPC increased with PSP; this relationship follows arithmetically from the constant loss and is not treated as an independent result.

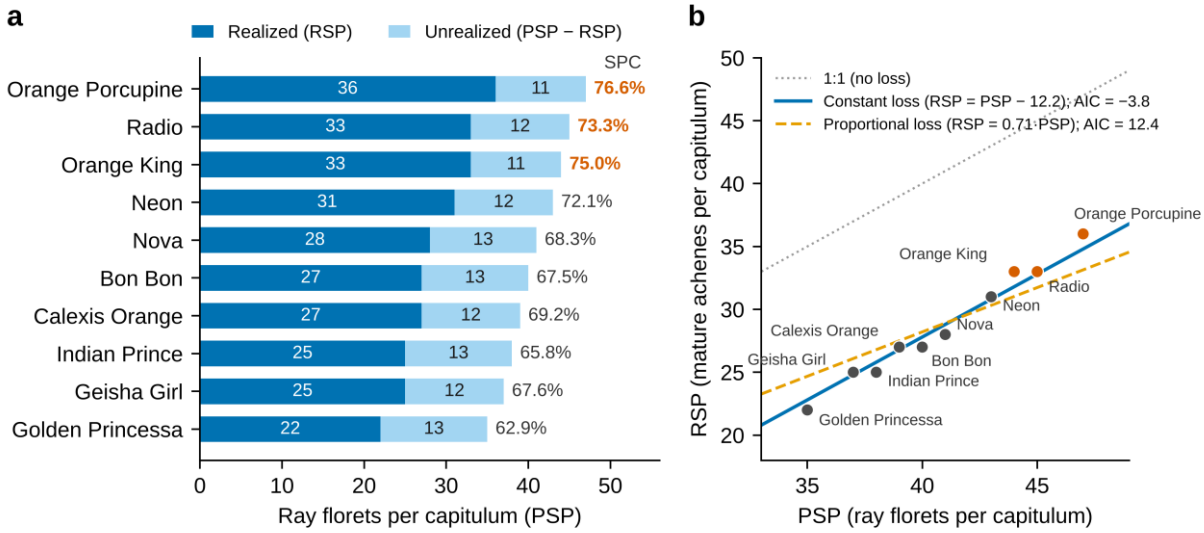

Fig. 1. (a) Realized (RSP) and unrealized ray florets per capitulum in ten *C. officinalis* cultivars, ordered by potential seed productivity (PSP); SPC is shown to the right of each bar. (b) Relationship between PSP and RSP across cultivar means (n=10) with fitted constant-loss and proportional-loss models; the dotted line marks complete realization. Highlighted points: 'Orange Porcupine', 'Orange King' and 'Radio'.

3.3. Plant-level seed output

Mature achenes per plant ranged from 1,762 in 'Golden Princessa' to 2,286 in 'Radio', followed by 'Orange King' (2,167) and 'Orange Porcupine' (2,130; Table 3). Plant RSP varied among cultivars about half as much as capitulum RSP (CV 7.1 vs 15.3%) and was positively associated with it (Spearman ρ= 0.89). The estimated number of seed-bearing capitula ranged from about 59 in 'Orange Porcupine' to about 82 in 'Indian Prince'. Cultivars with small capitula thus tended to compensate partly through more capitula; because the capitulum number was estimated from the two RSP values, this pattern is indicative and requires direct counts.

Table 3. Plant-level seed output of ten *C. officinalis* cultivars (Tashkent Region, 2023-2024).

Cultivar	Mature achenes plant^{-1} (counted)	Seed-bearing capitula plant^{-1} (estimated)
Radio (reference)	2,286	69
Bon Bon	1,984	73
Orange King	2,167	66
Golden Princessa	1,762	80
Indian Prince	2,058	82

Cultivar	Mature achenes plant^{-1} (counted)	Seed-bearing capitula plant^{-1} (estimated)
Nova	2,100	75
Neon	2,090	67
Orange Porcupine	2,130	59
Geisha Girl	1,896	76
Calexis Orange	2,012	75
Mean ± SD	2,049 ± 146	72.2 ± 7.0

Achenes were counted on 10 plants per cultivar. Capitula per plant = achenes per plant / achenes per capitulum (Table 2); this is a derived estimate, not an independent count.

3.4. Seed quality of the harvested seed

Germination energy ranged from 30 to 85%, germination from 54 to 96% and 1,000-seed mass from 9.9 to 12.0 g (Table 4). 'Orange King' (96%), 'Radio' (92%) and 'Orange Porcupine' (90%) had the highest germination, and 'Calexis Orange' (54%), 'Golden Princessa' (55%) and 'Indian Prince' (57%) the lowest. Germination increased with capitulum SPC ($\rho = 0.77$, $P = 0.009$; 95% bootstrap CI 0.21-1.00), as did germination energy ($\rho = 0.84$, $P = 0.002$; CI 0.35-1.00; Fig. 2). Because PSP, RSP and SPC are themselves closely related (§3.2), these associations cannot be attributed to any one of the three traits.

Table 4. Germination energy, germination and 1,000-seed mass of seed harvested from ten *C. officinalis* cultivars (2023-2024).

Cultivar	Germination energy (%)	Germination (%)	1,000-seed mass (g)
Radio (reference)	84	92	10.5
Bon Bon	60	65	10.2
Orange King	80	96	12.0
Golden Princessa	30	55	10.4
Indian Prince	48	57	10.2
Nova	63	76	10.3
Neon	70	85	10.3
Orange Porcupine	85	90	10.8
Geisha Girl	70	80	9.9
Calexis Orange	45	54	10.4

Germination: four replicates of 100 seeds (GOST 12038-84); 1,000-seed mass according to GOST 12042-80.

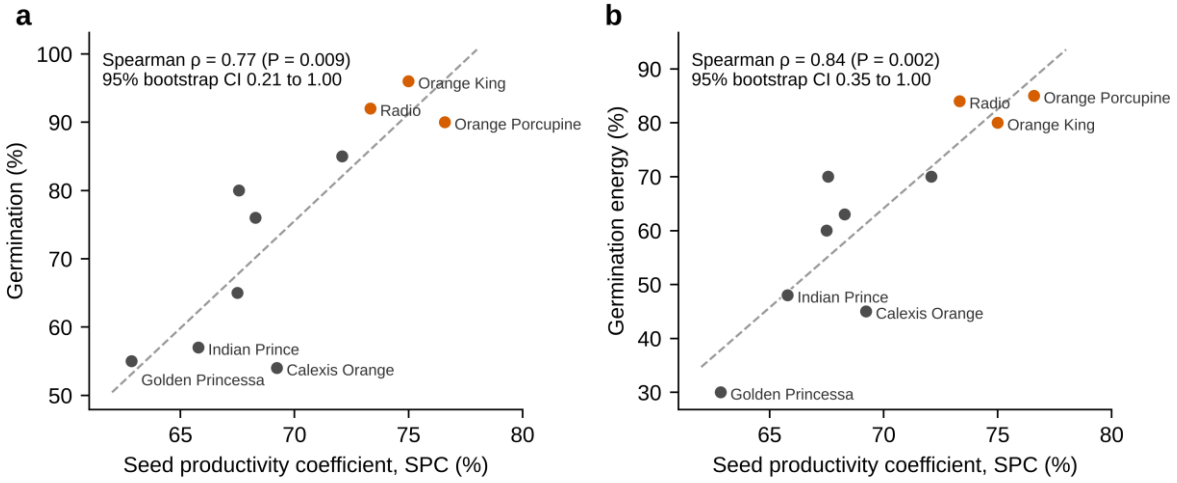

Fig. 2. Relationships between capitulum seed productivity coefficient (SPC) and (a) germination and (b) germination energy of the harvested seed across cultivar means (n = 10). Dashed lines are least-squares fits shown for orientation only.

3.5. Multivariate summary

The first principal component explained 80.5% of the variance among cultivars, and all seven traits loaded positively on it (loadings 0.26-0.41; Fig. 3b). It therefore represents a general axis of seed-production performance. ‘Orange Porcupine’ (PC1 score 3.13), ‘Orange King’ (3.05) and ‘Radio’ (2.61) were clearly separated from ‘Neon’ (1.04) and from the six cultivars with negative scores, the lowest being ‘Golden Princessa’ (−3.87; Fig. 3a). The second component (10.1%) was dominated by 1,000-seed mass (loading 0.93) and set ‘Orange King’, which had the heaviest seed, apart from the other two leading cultivars. The standardized trait profiles (Fig. 3c) show that the three leading cultivars combined above-average values for almost every trait.

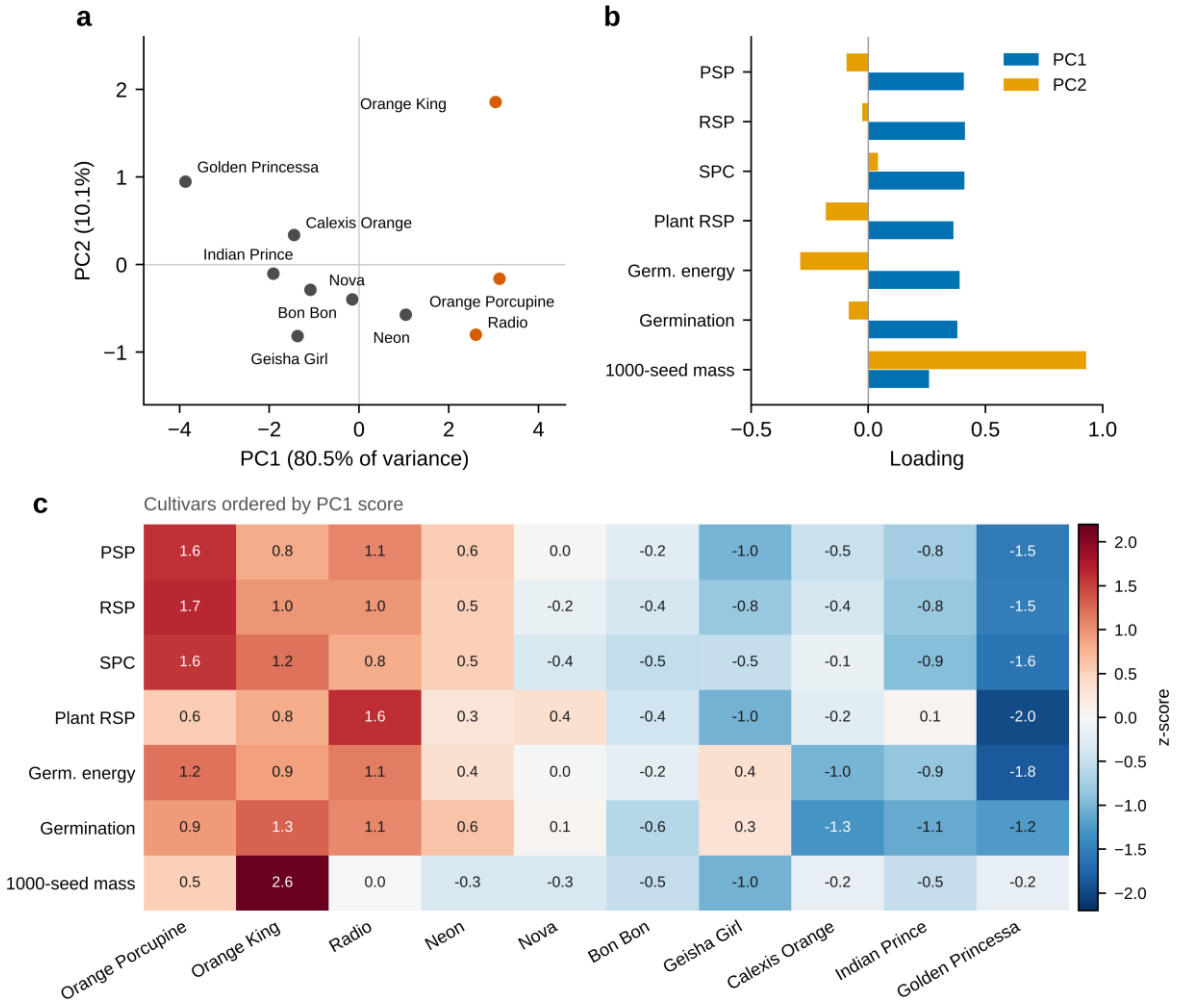

Fig. 3. Principal component analysis of seven standardized seed-production traits in ten *C. officinalis* cultivars. (a) Cultivar scores on PC1 and PC2; (b) trait loadings; (c) standardized trait values (z-scores), with cultivars ordered by PC1 score.

4. Discussion

The central result is that the number of ray florets that failed to set seed was nearly constant across cultivars, so that capitulum size and seed-set efficiency were not independent. A constant absolute loss is consistent with positional effects within the inflorescence, in which florets at particular positions or developing last are least likely to set seed because of resource competition or late pollination [12-14]. In calendula, achene form and size change systematically from the outer to the inner rows of the capitulum [8], which makes a position-dependent loss plausible. This interpretation was not tested here and requires counts of unrealized florets by row. If confirmed, it implies that within this cultivar set, selection for many ray florets per capitulum also selects for high seed-set efficiency.

The SPC values found here (63-77%) are higher than the realization of about 42% reported for a single cultivar in the Perm region [4]. The two values are not directly comparable, because the Perm estimate was made per plant and ours per capitulum, and the studies differ in cultivar, climate and management. The comparison nevertheless suggests that realization efficiency may depend strongly on environment. Under irrigation, water supply in the Tashkent Region did not depend

on rainfall, and apart from substantial rain in May 2024 (70.3 mm), the main seed-setting period from June to September was almost rainless in both years (Table 1). Such conditions may favour pollination and seed development, but this should be tested with weather records at flowering and replicated years.

At the plant level, cultivar differences were damped: plant RSP varied about half as much as capitulum RSP. Cultivars with small capitula appeared to compensate partly by producing more of them, in line with reports that seed yield of calendula depends strongly on the number of flower heads [2, 5]. Because capitulum number was estimated rather than counted, the strength of this compensation cannot be quantified from the present data, and conclusions about which cultivar produces the most seed per plant (here ‘Radio’) require direct counts on marked plants.

Germination of the harvested seed was higher in cultivars with higher capitulum PSP, RSP and SPC. Germination also depends on seed maturity at picking, drying and storage [2, 5], which were not controlled separately, so the association should not be read as a causal link between seed-set efficiency and seed viability. The PCA shows, however, that reproductive and seed-quality traits varied together along a single dominant axis. This supports evaluating them jointly when screening cultivars for seed production, an approach also used in cultivar comparisons in Western Siberia [11].

Limitations. Each cultivar occupied one unreplicated plot at one site, and capitula were sub-samples of that plot; cultivar differences therefore cannot be separated from plot effects, and no significance tests among cultivars were possible [15]. Values are pooled over two seasons that differed greatly in spring rainfall, so the year effect could not be assessed. Integer cultivar means limit the resolution of the floret-loss estimate. Capitulum number per plant was estimated, achene morphs and unrealized florets by row were not recorded, and all correlations rest on ten cultivar means.

Practical implication. ‘Orange Porcupine’, ‘Orange King’ and ‘Radio’ combined large capitula, high seed-set efficiency, high plant-level seed output and good germination, and ‘Orange King’ also produced the heaviest seed. These cultivars should be carried into replicated, multi-year seed-production trials that include direct capitulum counts per plant, counts of achene morphs and unrealized florets by row, and year-by-year analysis.

5. Conclusions

1. Capitulum PSP ranged from 35 to 47 ray florets, RSP from 22 to 36 mature achenes and SPC from 62.9 to 76.6% among ten cultivars; these differences are descriptive because plots were not replicated.

2. About 12 ray florets per capitulum failed to set seed in every cultivar. A constant-loss model fitted the data far better than a proportional-loss model, so larger capitula were also more efficient.

3. Mature achenes per plant (1,762-2,286) varied about half as much among cultivars as achenes per capitulum, indicating partial compensation through capitulum number, which needs direct verification.

4. Germination of the harvested seed (54-96%) increased with capitulum SPC in an exploratory analysis (ρ=0.77), and one principal component (80.5%) captured the joint variation of reproductive and seed-quality traits.

5. ‘Orange Porcupine’, ‘Orange King’ and ‘Radio’ are proposed as candidates for replicated, multi-year seed-production trials.

Declarations

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