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Floral biology and seed setting in standard carnation (*Dianthus caryophyllus*)

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ABSTRACT

Carnation (*Dianthus caryophyllus* L.) is one of the most popular cut flower crop grown under polyhouse. The present investigation was undertaken to assess the floral morphology, floral biology, pollen viability and seed setting behaviour among 12 genotypes at ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bengaluru during 2011-12. Results revealed that the genotype IIHRCG 201 recorded maximum stalk length, IIHRCG 207 recorded maximum flower diameter and flower length and IIHRCG 209 recorded highest vase life and flower duration. The genotype IIHRCG 207 with superior flower quality recorded maximum number of styles/pistil. The genotypes IIHRCG 210, IIHRCG 204, IIHRCG 209 and IIHRCG 201 recorded maximum duration of stigma receptivity. The genotypes IIHRCG 210 and IIHRCG 202 were found to be superior for number of pollen grains and percentage of stained pollen grains indicating their suitability as good pollen parents. Highest pollen germination was recorded in genotype IIHRCG 202 at 1, 2 and 3 hr of incubation period. The genotype IIHRCG 202 was found highly superior as pollen parent followed by IIHRCG 208 and IIHRCG 210. On selfing and natural cross pollination, seed setting was not observed in all the 12 genotypes studied. On artificial cross pollination, seed setting was observed in four genotypes namely IIHRCG 202, IIHRCG 204, IIHRCG 205 and IIHRCG 210. Earliest capsule maturity was recorded in genotype IIHRCG 210 followed by IIHRCG 203 and IIHRCG 208. Highest number of seeds/capsule was recorded in IIHRCG 202 followed by IIHRCG 204 and IIHRCG 210.

Key words: Carnation, Evaluation, Floral biology, Seed setting

Carnation (*Dianthus caryophyllus* L.) is an important flower crop having great commercial value as a cut flower due to its excellent keeping quality, wide array of colour and forms. It is grown commercially in India in places having mild climate like Solan, Shimla, Kalimpong, Kodaikanal, Mandi, Kullu, Srinagar, Ooty and Yercaud. In Pune and Bengaluru, it is grown under controlled condition. In Karnataka apart from Bengaluru, the entire part of transitional belt seems to be very ideal for cultivation of flowers on account of favourable climate, soil and other factors (Shiragur 2004b). Carnation (*Dianthus caryophyllus* L.) belongs to the family Caryophyllaceae having diploid chromosome number 2n=30. Dong Lian Xin *et al.* (2009) reported that genus *Dianthus* containing 300 species distributed mainly in Europe and Asia.

Evaluation of genotypes helps the breeder to select agronomically desired genetic back ground for crop improvement. Studying the extent of variability for economically important traits, floral, morphological and biology characters could facilitate the breeder in making prudent choice of parents for hybridization. Identification of superior pollen parents based on quantity and viability of pollen decides the extent of success the breeder achieves. Presence of variable ploidy types, lack of sufficient and viable pollen among cultivated varieties, cross incompatibility, chromosomal aberrations resulting in the formation of very few viable gametes, post-zygotic barriers at immature stage, formation of embryo without endosperm and incompatibility are the major impediments to breeders for speedy evolution of indigenous varieties in carnation. Considering the above facts, three experiments were carried out with following objectives: (i) Evaluation of genotypes for vegetative, floral morphology and biology traits. (ii) Identification of pollen parents through pollen viability. (iii) Assessment of genotypes for seed setting.

MATERIALS AND METHODS

Three sets of experiments were carried out in a naturally ventilated polyhouse (200 m^2) at ICAR-Indian Institute of Horticultural Research, Hesarghatta Lake Post, Bengaluru during 2011-12. The experimental site was located at 13°58° N latitude and 77°37° E longitude with an altitude of 890 meters above mean sea level. The experiment was laid out with 12 genotypes, viz. IIHRCG 199, IIHRCG 200, IIHRCG 201, IIHRCG 202, IIHRCG 203, IIHRCG 204, IIHRCG 205, IIGRCG 206, IIHRCG 207, IIHRCG 208, IIHRCG 209 and

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Table 1 Petal margin and flower colour of genotypes used in the study

Genotype	Petal margin	Flower colour
IIHRCG 199	Serrated	Major petal area: Green yellow group 2D Petal margin: Red group 39B
IIHRCG 200	Serrated	Red purple 58B
IIHRCG 201	Serrated	Red-group 44B
IIHRCG 202	Serrated	Major petal area: White group 155A Petal margin: Red purple 62A
IIHRCG 203	Serrated	Red group 43D
IIHRCG 204	Serrated	Red group 36C
IIHRCG 205	Serrated	Red group 40A
IIHRCG 206	Serrated	Major petal area: Orange group 27A Petal margin: Orange red group 33A
IIHRCG 207	Serrated	Major petal area: Red group 36APetal margin: Red group 39B
IIHRCG 208	Serrated	White group 155D
IIHRCG 209	Serrated	White group 155D
IIHRCG 210	Serrated	Major petal area: White group 159DPetal margin: Red purple group 61B

IIHRCG 210 in CRD with three replication. The recommended package and practices were followed throughout the experiment. Flower colour and petal margin of 12 genotypes is given in Table 1.

All the three experiments were laid out in CRD with three replication.

The observations recorded for vegetative traits were stalk length (cm), number of nodes/shoot, inter-nodal length (cm), length and breadth of 5th leaf (cm); traits recorded for floral morphology were flower diameter (cm), bud length (cm), flowering duration (days), number of petals/flower, vase life (days), flower colour (RHS colour chart) and petal margin; traits recorded for floral biology were number of styles/pistil, number of stamens/flower, days to anthesis, duration of stigma receptivity (hr), anther dehiscence in three stages of flower development. Presence of anther dehiscence was observed using hand magnification lens in all the three different stages of flower development from 10 tagged flowers for each stage.

The Experiment 2 was conducted in laboratory condition in CRD with three replication. Observations were recorded on total number of pollen grains, stained pollen grains (%), round shaped pollen grains (%) and pollen germination after 1, 2 and 3 hr of incubation from 10 microscopic fields per replication at 40X magnification of a compound microscope.

Basic pollen germination medium by Brew Baker and Kwack (1963) was modified based on the earlier reports of pollen germination in *Dianthus* and related species. The modified media composition used for pollen germination consisted of sucrose (8%) supplemented with calcium nitrate [$(NO_3)_4$. $2H_2O$] (100 ppm), magnesium sulphate ($MgSO_4.7H_20$) (325 ppm), potassium nitrate ($KN0_3$) (200 ppm) and boric acid (150 ppm). For incubation, petri dish was kept in culture room for 3 hr at 25°C.

The pollen germination was carried out following hanging drop technique using cavity slides as suggested by Stanely and Linkens (1974). The pollen grains were placed in small drops of the solution on a cover slip. The cover slip was then inverted over the cavity slides taking care that the drop did not touch the cavity. The margins of the cover slip were smeared with grease to check evaporation and drying out of germinating media. The slides were placed in petri dishes having moist filter paper at the bottom and were covered with moistened cloth to ensure lasting uniform humidity.

Observations on seed setting related traits were recorded from flowers subjected for self pollination, natural cross pollination and artificial cross pollination. Days to capsule maturity, number of seed setting flowers and number of seeds/capsule were recorded. In self pollination, the well developed buds were marked. On the day of stigma maturity hand self-pollination was accomplished by direct transfer of pollen with the help of a camel hair brush from freshly dehisced anthers to the stigma of flower of the same plant. In natural cross pollination, flower buds were allowed to develop capsules under natural conditions from neighbouring plants of different genotypes. In artificial cross pollination, flower buds were emasculated 24 hr before anthesis and the pollen of selected genotypes was collected in petri-plate and kept under sunlight for one to two hours. After the bursting of pollen grains these were taken in the petri-plate and were used for pollination. Pollination was repeated three times in subsequent days for three days. The flowers thus pollinated were shielded from unwanted pollen both before and after pollination by covering with butter paper bags.

RESULTS AND DISCUSSION

The data obtained from each of the experiment were subjected to statistical analysis. Results obtained are presented and discussed under following headings.

Experiment 1: Evaluation of genotypes for vegetative, floral morphology and biology traits

Evaluation of genotypes help the breeder to select genotypes with desired genetic background for undertaking crop improvement activities aimed at evolving superior cultivars. On the perusal of the data presented in Table 2 indicated that all the vegetative parameters except stalk length and width of 5th leaf are significantly influenced by different genotypes. Six genotypes, viz. IIHRCG 200, IIHRCG 201, IIHRCG 203, IIHRCG 205, IIHRCG 207 and IIHRCG 209 recorded stalk length exceeding 50 cm. The genotypes with stalk length of 50 cm fetch more prices in the market. Variation in stalk length has also been reported by Singh and Sangama (2003). The number of nodes ranged from 8.33 (IIHRCG 210) to 14.67 (IIHRCG 201 and IIHRCG 203). The maximum inter-

Table 2 Performance of twelve carnation genotypes for vegetative traits

Genotype	Stalk length (cm)	Number of nodes	Internodal length (cm)	Length of 5th leaf (cm)	Width of 5th leaf (cm)
IIHRCG 199	41.17	14.33	4.74	7.48	0.66
IIHRCG 200	53.00	11.00	7.48	8.42	0.62
IIHRCG 201	56.70	14.67	6.10	10.99	0.62
IIHRCG 202	49.57	9.33	5.00	6.97	0.68
IIHRCG 203	51.53	14.67	5.71	7.59	0.51
IIHRCG 204	48.47	11.67	8.12	8.12	0.58
IIHRCG 205	52.20	8.67	5.76	8.33	0.65
IIHRCG 206	47.87	12.67	5.90	8.06	0.70
IIHRCG 207	50.53	9.33	5.12	8.78	0.66
IIHRCG 208	46.90	12.33	6.32	9.12	0.65
IIHRCG 209	50.30	14.33	6.00	8.39	0.65
IIHRCG 210	43.17	8.33	8.30	11.8	0.61
F test	NS	**	**	**	NS
SEm ±		0.84	0.42	0.42	
CD (P=0.05)		2.46	1.24	1.24	

NS, Nonsignificant

Table 3 Performance of twelve carnation genotypes for floral traits

Genotype	Flower diameter (cm)	Bud length (cm)	Flower duration (cm)	Number of petals	Vase life (days)
IIHRCG 199	6.91	5.11	9.00	62.00	9.00
IIHRCG 200	6.16	4.75	9.66	72.00	9.66
IIHRCG 201	6.26	4.86	10.66	56.00	10.66
IIHRCG 202	4.56	4.67	9.00	57.66	9.00
IIHRCG 203	6.38	5.20	10.66	47.33	10.66
IIHRCG 204	6.03	4.79	10.66	51.00	10.66
IIHRCG 205	6.66	4.97	10.00	60.66	10.00
IIHRCG 206	5.83	4.98	9.00	63.66	9.00
IIHRCG 207	7.38	5.30	11.66	70.33	11.66
IIHRCG 208	6.21	5.15	11.33	48.33	11.33
IIHRCG 209	5.80	5.23	12.00	58.66	12.00
IIHRCG 210	5.86	5.43	10.33	57.66	10.33
F test	**	NS	NS	NS	**
SEm±	0.32				0.61
CD (P=0.05)	0.94				1.78

NS, Nonsignificant

nodal length was recorded in IIHRCG 210 (8.30 cm) while, lowest recorded in IIHRCG 199 (4.74 cm). Inter-nodal length and number of nodes have direct influence on flower stalk length. Similar trends were also reported by Shiragur *et al*. (2004a) and Ryagi *et al*. (2007). The genotype IIHRCG 210 recorded maximum length of 5th leaf (11.80 cm), followed by IIHRCG 201 (10.99 cm) and lowest leaf length was recorded in IIHRCG 202 (6.97 cm).

Significant variation across the genotypes was recorded for flower diameter and vase life, however, parameters such as bud length, flower duration and number of petals were found to be non-significant (Table 3). The maximum flower diameter was recorded IIHRCG 207 (7.38 cm), while, it was minimum in IIHRCG 202 (4.56 cm). Sathisha (1997) and Naveen Kumar *et al*. (1999a/b) also reported variation in flower diameter in different genotypes. Highest vase life was recorded in IIHRCG 209 (12 days) while, lowest recorded in IIHRCG 199, IIHRCG 202 (9 days) and IIHRCG 206 (9 days). The flower duration ranged from 9 days (IIHRCG 199, IIHRCG 202 and IIHRCG 206) to 12 days (IIHRCG 209). The genotype IIHRCG 209 (12 days) which had highest vase life also recorded maximum flower duration probably indicating the direct positive association between them. For the improvement of these traits, selection for one will suffice. Singh and Sangama (2003) reported similar results for vase life in 'Sunrise' (12.60 days) followed by 'Forca' (12.40 days).

Data pertaining to floral traits are presented in Table 4. The genotype IIHRCG 207 with superior flower quality recorded maximum number of styles per pistil and was on par with other genotypes with respect to number of stamens per flower. It could be inferred that IIHRCG 207 was found to be superior as a best parent for hybridization as it possess better flower quality and adequate quantity of pollen. Genotypes with higher stigma receptivity enable breeder to synchronize crossing programme based on anthesis, time of anther dehiscence and anther dehiscence in relation to different stages of flower development. Information on anther maturity and stigma receptivity also indicate the status of dichogamy, viz. protandry and protogyny. Highly significant differences were observed for number of styles per pistil among the genotypes, which was ranged from 2.50 (IIHRCG 204) to 4.16 (IIHRCG 205 and IIHRCG 207). The differences among genotypes for

Table 4 Evaluation of twelve carnation genotypes for floral biology traits

Genotype	Number of styles/ pistil	Number of Stamens	Days to anthesis	Duration of stigma receptivity (hr)
IIHRCG 199	2.83	14.83	4.33	48.00
IIHRCG 200	3.50	5.66	6.00	59.00
IIHRCG 201	3.83	10.33	4.33	63.00
IIHRCG 202	3.00	4.33	3.67	55.00
IIHRCG 203	2.83	9.83	4.33	43.00
IIHRCG 204	2.50	10.16	5.67	70.00
IIHRCG205	4.16	8.00	3.00	63.00
IIHRCG 206	3.83	6.50	3.33	51.00
IIHRCG 207	4.16	8.16	4.33	55.00
IIHRCG 208	3.88	5.16	4.67	65.00
IIHRCG 209	3.33	7.50	4.33	70.00
IIHRCG 210	3.50	8.50	4.00	73.00
F test	**	NS	**	**
SEm±	0.29	2.09	0.34	4.07
CD (P=0.05)	0.86		1.01	12.00

NS, Nonsignificant

number of stamens were statistically non-significant, however, maximum number of stamens were recorded in IIHRCG 199 (14.83) followed by IIHRCG 201 (10.33) and IIHRCG 204 (10.16), while it was recorded minimum in IIHRCG 202 (4.33). Highly significant differences were recorded for days to anthesis which was ranged from 3 days (IIHRCG 205) to 6 days (IIHRCG 200).The difference between IIHRCG 200 (6.00) and IIHRCG 204 (5.67) was statistically on par. Maximum duration of stigma receptivity was recorded in IIHRCG 210 (73.00 hr) followed by IIHRCG 204 (70 hr), IIHRCG 209 (70 hr), IIHRCG 208 (65.00 hr) and IIHRCG 201 (63.00), while IIHRCG 203 recorded minimum duration of stigma receptivity (43.00 hr). Gupta *et al*. (2004) reported maximum flower anthesis and pollen dehiscence between 10-12 scaps and artificial pollination 2 days after anthesis in *Dianthus barbatus.*

Visual observations on anther dehiscence in three different stages of flower development are presented in Table 5. Of the 12 genotypes observed, 7 and 11 witnessed anther dehiscence in bud and half opened flowers, respectively. The genotypes IIHRCG 202, IIHRCG 204, IIHRCG 205, IIHRCG 207 and IIHRCG 210 recorded anther dehiscence at fully opened flowers (anthesis). Two third flowers of 5 and 6 genotypes exhibited anther dehiscence at bud and half opened flowers, respectively. Stigma receptivity was not noticed in all the three flower stages selected for visual observation of anther dehiscence indicating protandry and allogamy. This will facilitates breeders to identify right time of pollen collection from different genotypes when these are used as pollen parents and also help in designing a systematic crossing programme. Shafi Bhat *et al*. (1991) reported anthesis at 6 scaps with the highest number of flowers (31.98%) opening between 9 scaps and 10 scaps, and anther dehiscence commenced immediately after anthesis. Dhaduk *et al.* (1987) reported heterostyly and pentaploidy condition in gladiolus.

Table 5 Visual observations on anther dehiscence at different stages of flower development

Genotype	Bud	Half opened	Full opened
IIHRCG 199	+	++	-
IIHRCG 200	++	+	-
IIHRCG 201	++	+	-
IIHRCG 202	-	-	+++
IIHRCG 203	++	+	-
IIHRCG 204	-	++	+
IIHRCG 205	-	++	+
IIHRCG 206	++	+	-
IIHRCG 207	-	++	+
IIHRCG 208	++	+	-
IIHRCG 209	+	++	-
IIHRCG 210	-	++	+

- = Anther dehiscence not observed, + = Anther dehiscence observed in 1/3rd of the flowers, ++ = Anther dehiscence observed in 2/3rd of the flowers, +++ = Anther dehiscence observed in all the flowers observed.

Experiment 2: Identification of pollen parents through pollen viability

Pollen staining and pollen shape: Significant variations observed for number of pollen grains, stained pollen grains and round shaped pollen grains (Table 6). Identification of superior pollen parents based on quantity and viability decides the extent of success the breeder achieves in any systematic hybridization programme. Study on pollen viability through pollen staining and shape gives a crude assessment of viability of pollen grains of a particular genotype. Though the genotype IIHRCG 207 recorded highest number of stamens/flower, similar trend was not recorded with respect to total number of pollen grains and percentage of stained pollen grains. The genotypes IIHRCG 210 and IIHRCG 202 were found to be superior for number of pollen grains and percentage of stained pollen grains indicating their suitability as good pollen parents. Shafi Bhat *et al*. (1993) reported pollen viability as ascertained by a staining test ranged from 89.18% for fresh pollen to 31.94% after 10 days of storage under laboratory conditions (20-25^{o}C). Dafni and Firmage (2000) opined that consideration of pollen viability is important in studies on pollen storage, reproductive biology and hybridization.

The genotype IIHRCG 208 which was statistically on par with IIHRCG 210 and IIHRCG 202 for number of pollen grains and percentage of stained pollen grains also recorded highest percentage of round shaped pollen grains. It can be inferred that, while selecting genotypes as pollen parents in addition to number and staining of pollen grains, breeders need to consider shape of the pollen grains. Shivanna and Mohan Ram (1993) attributed variability in the quality of collected pollen to changes in environmental conditions, genotypic differences, vigour and physiological status of the plant. In many ornamental species, the cut flower inflorescences continue to produce viable pollen for several days, provided the cut ends are dipped in a container with

Table 6 Stained and round shaped pollen grains in twelve genotypes of carnation

Genotype	Number of pollen grains	Stained pollen grains (%)	Round shaped pollen grains (%)
IIHRCG 199	6.89	16.62 (23.79)	26.19 (30.66)
IIHRCG 200	5.89	60.31 (51.02)	71.12 (57.56)
IIHRCG 201	4.89	24.57 (29.43)	33.82 (35.38)
IIHRCG 202	7.22	61.90 (51.93)	67.64 (55.52)
IIHRCG 203	5.67	31.23 (33.94)	52.01 (46.14)
IIHRCG 204	5.22	25.92 (30.18)	40.61 (39.19)
IIHRCG 205	6.55	32.60 (34.56)	52.27 (46.28)
IIHRCG 206	5.99	35.37 (36.36)	40.65 (39.45)
IIHRCG 207	5.78	28.52 (32.08)	79.35 (63.01)
IIHRCG 208	9.66	37.57 (37.79)	79.54 (636.20)
IIHRCG 209	5.89	46.24 (42.63)	44.53 (41.63)
IIHRCG 210	7.33	66.94 (55.35)	65.35 (54.19)
SEm±	0.76	5.99	6.80
CD (P=0.01)	2.24	14.98	16.35

distilled water (Viehmeyer and Uhlinger 1971).

Pollen germination: There were highly significant variations recorded for total number of pollen grains, pollen grains germinated (%) after one hour, two hours and three hours among the genotypes evaluated (Table 7). Though, breeder uses the information on shape, quantity and staining of pollen grains as indicators of assessing pollen viability. However, many contradictory reports are available in different crops indicating poor success in hybridization. In this view, pollen germination was conducted to ascertain the viability of pollen grains while selecting superior pollen parents. Highest pollen germination was recorded in IIHRCG 202 at all the three incubation period i.e. 1 hr after, 2 hr and 3 hr with a mean of 40.19%, 50.11% and 47.28%, respectively. Shafi Bhat *et al*. (1993) reported highest pollen grain germination (27.73%) in *Dianthus caryophyllus* at 4% sucrose solution; Gupta *et al*. (2004) reported highest pollen germination at 6% sucrose media in *Dianthus barbatus*.

Experiment 3: Assessment of genotypes for seed setting

Results pertaining to artificial cross pollination are presented in Table 8. Selfing and natural cross pollination recorded no seed setting in all the 12 genotypes. On artificial cross pollination, seed setting flowers were recorded in four genotypes namely IIHRCG 202, IIHRCG 204, IIHRCG 205 and IIHRCG 210. However, the genotype IIHRCG 210 recorded highest seed setting flowers (22.22%). Gupta *et al*. (2004) have found best capsule set on artificial pollination in *Dianthus barbatus*.

Earliest capsule maturity was recorded in IIHRCG 210 (41 days) followed by IIHRCG 203 (46 days) and IIHRCG 208 (47 days). Gupta *et al*. (2004) reported formation of capsule took 11 days from day of flower anthesis in *Dianthus barbatus*. Highest number of seeds per capsule was recorded in IIHRCG 202 (30) followed by IIHRCG 204 (8) and IIHRCG 210 (7). Gupta *et al*. (2004) found artificial pollination as the best method of pollination. The results on pollen germination and seed setting on artificial cross pollination found that IIHRCG 202 and IIHRCG 201 as superior seed and pollen parents. This could be attributed probably to compatible ploidy either diploid or tetraploid background, male and female fertility and absence of incompatibility. The same need to be further corroborated with cytological investigations.

Table 7 Percentage pollen germination in twelve genotypes of carnation

Genotype	Number of pollen grains	Percentage of pollen grains germinated		
		After one hour	After two hours	After three hours
IIHRCG 199	17.55	20.76 (12.74)	21.23 (13.22)	22.48 (14.96)
IIHRCG 200	15.77	40.15 (41.64)	41.54 (44.07)	44.18 (48.60)
IIHRCG 201	4.11	36.14 (34.92)	36.14 (34.92)	36.14 (34.92)
IIHRCG 202	6.33	40.19 (41.72)	50.11 (58.78)	47.28 (54.01)
IIHRCG 203	2.66	26.24 (19.76)	30.46 (26.43)	31.36 (27.30)
IIHRCG 204	6.44	32.56 (29.02)	32.56 (29.02)	35.27 (33.46)
IIHRCG 205	5.11	32.36 (28.83	33.76 (31.21)	32.36 (28.83)
IIHRCG 206	5.55	38.00 (37.96)	38.00 (37.96)	41.60 (44.13)
IIHRCG 207	6.77	24.64 (17.75)	24.64 (17.75)	28.58 (22.92)
IIHRCG 208	4.22	34.49 (33.20)	34.49 (33.20)	44.52 (49.15)
IIHRCG 209	10.66	32.05 (28.26)	32.74 (29.37)	32.05 (28.26)
IIHRCG 210	12.11	38.07 (38.15)	40.97 (43.07)	42.67 (46.02)
SEm±	1.19	2.93	3.46	2.93
CD (P=0.01)	4.72	11.59	13.69	11.60

Figures in parenthesis are the angular transformed means

Table 8 Performance of carnation genotypes for seed setting on artificial cross pollination

Genotype	Seed setting flower (%)	Days taken for capsule maturity	Number of seeds
IIHRCG 199	0.00	56	0
IIHRCG 200	0.00	59	0
IIHRCG 201	0.00	45	0
IIHRCG 202	11.11	49	30
IIHRCG 203	0.00	46	0
IIHRCG 204	11.11	58	8
IIHRCG 205	11.11	54	4
IIHRCG 206	0.00	48	0
IIHRCG 207	0.00	52	0
IIHRCG 208	0.00	47	0
IIHRCG 209	0.00	45	0
IIHRCG 210	22.22	41	7

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