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Genetic advance for physiological parameters in two high yielding castor (*Ricinus communis* L.) genotypes under irrigation and moisture stress

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Abstract

During Kharif-2014, a field study was conducted with two high yielding CRIDA castor genotypes, viz., CRC-2 and CRC-9 along with the check 48-1 under irrigated and moisture stress conditions to assess their variability and genetic advance for seed yield and physiological traits viz., ELWRC, RWC, Pn, gs, Tr and WUE. Significant variability was observed among the genotypes for all the characters studied. CRC-9 performed better than the check for the four physiological traits studied viz., ELWRC, Pn, gs and WUE under both irrigated and moisture deficit stress conditions. Whereas, CRC-2 performed better than the check for two physiological traits viz., gs and Tr under moisture deficit conditions only. High heritability with high genetic advance was recorded for Pn under both the conditions studied while for gs and Tr under moisture deficit stress only indicating the predominance of additive gene action in inheritance of these characters. Hence, these traits can be taken into consideration for the improvement of castor.

Keywords: Castor, genotypes, seed yield, physiological traits, genetic variability, genetic advance

Introduction

Castor bean (*Ricinus communis* L.) is a tropical non-edible oil plant of high commercial importance. Castor bean is monotypic species of the family Euphorbiaceae has a wide range distribution in tropical and subtropical regions (Dapke *et al.*, 2016) [16]. The major production of castor bean comes from India, China and Brazil, which contributes more than 90% of global production. The productivity of the country in 2011-12 is 1417 kg ha⁻¹ with world average of 850 kg ha⁻¹ (Anonymous, 2013) [2]. Gujarat is the leading castor growing state in India and contributes more than 70% of the production from about 47% of the area (Dapke *et al.*, 2016) [16].

While a full understanding of the physiological and genetic basis of yield is still incomplete, progress has been made in developing selection technologies that may improve the efficiency of experimental breeding and the physiological traits conferring survival were strongly favored for most of the crop's evolution (Reynolds *et al.*, 1999) [30]. Crop physiologists have identified a number of traits that would help the breeder in development and identification of moisture stress tolerant genotypes with high yield potential (Basu *et al.*, 2004) [5]. Apparently, morpho-physiological traits for growth and development have the greatest impact on the adaptation of plants to the target environments with the aim of achieving a maximum productivity (Rahman *et al.*, 2016) [27].

Materials and methods

During Kharif-2014, a field study was conducted with two CRIDA castor genotypes, viz., CRC-2, CRC-9 and a check 48-1 at Hayathnagar Research Farm, Central Research Institute for Dry land Agriculture (CRIDA), Hyderabad. The trial was sown on July 26th, 2014 in RBD with three replications each for irrigated conditions and for moisture deficit stress conditions with two meters apart of these two treatments. The irrigated plots were maintained stress free by withholding irrigation at initiation of primary moisture deficit stress treatment was imposed till wilting symptoms were appeared and then the stress was relieved. Five plants from each treatment were randomly selected in each replication of all the genotypes. Three replications were maintained in the field for each treatment. Each genotype was sown in 5m length of three rows with plant to plant spacing of 30cm and 1m between rows.

The crop received 249 mm rainfall spreading in 19 rainy days (>2.5 mm) during the crop growth period, the crop experienced rainless period of more than 10 days during vegetative stage, 31 days during initiation to maturation of primary and initiation of secondary and 97

days during maturation of secondaries and initiation to maturation of tertiaries. During the crop growth period, the average temperature was 30.3°C with minimum and maximum of 4.5°C and 35.6°C respectively.

Physiological Parameters

Photosynthetic rate (Pn), stomatal conductance (gs) and transpiration rate (Tr): A portable photosynthesis system (LI-6400, LI-COR, USA) was used to record photosynthetic rate (Pn- $\mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$), stomatal conductance (gs- cm s^{-1}), transpiration rate (Tr- $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) on fully expanded young leaves for each treatment between 9:30 and 12:00hrs. Intrinsic water use efficiency (WUE- $\mu\text{mol CO}_2 \text{mmol}^{-1} \text{H}_2\text{O}$) was calculated as the ratio of Pn and Tr i.e. $\text{WUE} = \text{Pn}/\text{Tr}$.

Relative water content (RWC): RWC was determined according to Barr and Weatherley (1962). Fresh weight of the young fully expanded leaf was taken and soaked the leaf for 4 hours and after soaking, the leaves were quickly and carefully blotted dry with tissue paper prior to determination of turgid weight. Finally, the samples were dried in an oven at 65 °C for 48 hours to determine dry weights.

$$\text{RWC} = [(\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight})] \times 100$$

Excised leaf water retention capacity (ELWRC): ELWRC was measured as method of Rao et al. (1998) [28]. Third leaf from top were excised and weights were taken at zero min, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360 minutes. Then curves were drawn to record the moisture percent actually retained by the leaf when it was excised from the plant and expressed in %.

$$\text{ELWRC} = [\text{initial weight at that specific time}/\text{Initial weight}] \times 100.$$

Genetic Analysis

- Heritability in broad sense (H^2 or h^2) (Falconer, 1989)
- Phenotypic and genotypic correlations (Miller *et al.*, 1958)
- Genotypic (σ^2_g) and Phenotypic variances (σ^2_p) (Comstock and Robinson, 1952).
- Phenotypic Coefficient of Variation (PCV) and Genotypic Coefficient of Variation (GCV) (Singh and Chaudhary, 1985).
- Statistical analysis - Analysis of variance (ANOVA)-

STAR (Statistical Tools For Agricultural Research)

Table 1: Weather data during crop growth period-Kharif-2014

	Temp		RH	
	Max. (°C)	Min. (°C)	Max (%)	Min (%)
Average	30.3	14.4	84.8	49.7
Minimum	21.3	4.5	42	24.0
Maximum	35.6	31	100.0	49.7

Total Rainfall = 249 mm Number of rainy days (>2.5 mm) = 19 days

Results and Discussion

Data on six physiological parameters *viz.*, photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (Tr), intrinsic water use efficiency (WUE), ELWRC and RWC were recorded for the two genotypes *viz.*, CRC-2 and CRC-9 along with the check 48-1 during Kharif-2014 under both irrigated and moisture stress conditions. The mean performances and percentage superiority (% sup.) of seed yield and physiological traits of the two genotypes over check under irrigated and moisture stress conditions are presented in Table-2(a) and Table-2(b) respectively.

Seed yield (g/pl): Under irrigated conditions, the seed yield was highest for CRC-2 (41.45g/pl) while for CRC-9 it was lower than the check recording 29.18 g/pl while the check 48-1 recorded 36.45g/pl [Table-2 (a)].

Under moisture stress condition also CRC-2 recorded the highest seed yield of 27.68g/pl while CRC-9 recorded 22.21 g/pl which was on par with the check 48-1 (22.57 g/pl). The % of superiority over check 48-1 for CRC- 2 was 13.72% and 22.64% under irrigated and moisture stress conditions respectively. Whereas, for CRC-9 the % superiority over check under irrigated conditions was inferior while under moisture deficit stress conditions it was on par with the check 48-1 [Table-2(b)].

Physiological components

Excised leaf water retention capacity (ELWRC): ELWRC values at the end of 420 min of excision of leaves was highest for CRC-9 (73.96%) followed by 48-1 (70.63%) and CRC-2 (64.57%). This results indicated that high ELWRC was retained by CRC-9 with better leaf moisture even after 420 min of excision whereas, CRC-2 and check 48-1 showed lower leaf moisture content for the same duration.

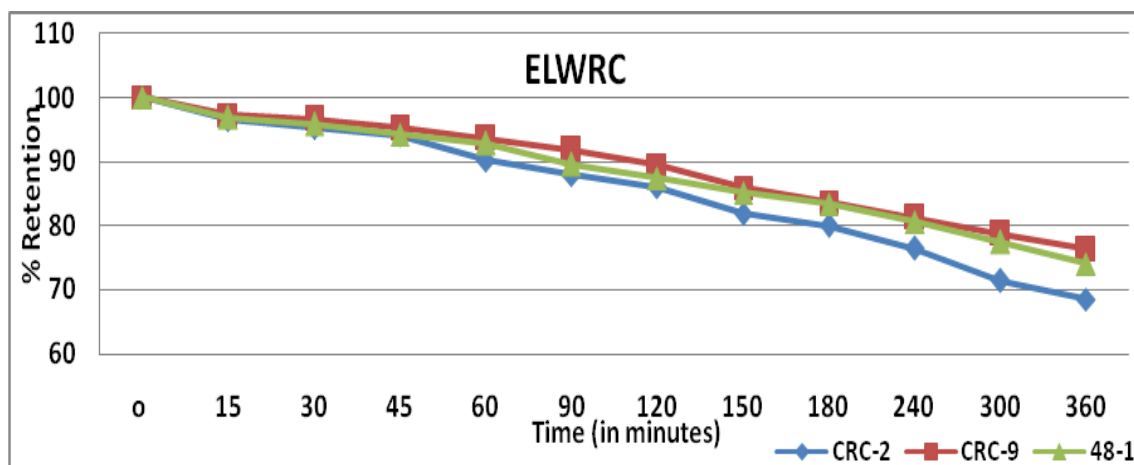


Fig 1: ELWRC of three castor genotypes

Relative water content (RWC): RWC was higher for CRC-2 with 73.89% under irrigated conditions while for CRC-9 it was 69.73% and for check it was 73.54% under irrigated conditions. While under moisture stress, RWC was higher for

check (48-1) with 69.68% followed by CRC-9 (64.69%) and CRC-2 (59.62%). These results showed that none of the genotypes studied were superior to the check under both the conditions.

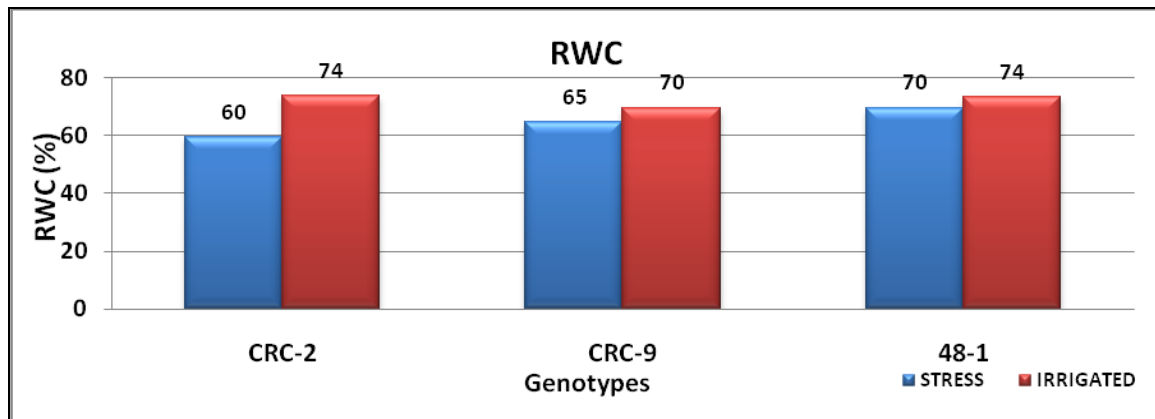


Fig 2: RWC of three castor genotypes under irrigated and moisture deficit stress conditions

Photosynthetic rate (Pn- $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$): Under irrigated conditions, CRC-9 ($39.57 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) recorded higher Pn values than CRC-2 ($33.7 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) followed by check 48-1 ($29.87 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). Under moisture deficit stress also CRC-9 recorded higher Pn values of $36.9 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ followed by CRC-2 ($28.77 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and the check 48-1 ($20.9 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). The results showed that % superiority over check for CRC-9 was 32.47% under irrigated conditions, while it was 76.56% under moisture deficit stress. Whereas, for CRC-2, it was 12.82% under irrigated conditions while under stress conditions it was 37.66%.

Stomatal conductance (gs- cm s^{-1}): The genotype CRC-9 (0.65 cm s^{-1}) showed higher gs than CRC-2 (0.58 cm s^{-1}) and 48-1 (0.45 cm s^{-1}) under irrigated conditions. Whereas, under moisture deficit stress, the two genotypes CRC-2 and CRC-9 showed similar gs of 0.42 cm s^{-1} but higher than 48-1 (0.21 cm s^{-1}). The results showed that % superiority over check for CRC-9 was 30% under irrigated conditions while it was 100% under moisture deficit stress conditions. Whereas, for CRC-2, it was 16% under irrigated conditions while under stress conditions it was 100%.

Transpiration rate (Tr- $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$): Tr was higher for CRC-9 with $9.61 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ followed by CRC-2 ($8.67 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and check 48-1. Under moisture deficit stress conditions, Tr was higher for CRC-2 ($8.08 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) than CRC-9 ($7.85 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and 48-1. The results showed that % superiority over check for CRC-9 was 17.77% under irrigated conditions while it was 51.84% under moisture deficit stress conditions. Whereas, for CRC-2, it was 6.25% under irrigated conditions while under stress conditions it was 56.29%.

Water use efficiency (WUE- $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$): Under irrigated conditions, CRC-9 recorded higher WUE of $4.12 \mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ than CRC-2 ($3.89 \mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$) and 48-1 ($3.66 \mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$). Under moisture deficit stress, CRC-9 recorded higher WUE of $4.70 \mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ followed by CRC-2 ($3.56 \mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$) and 48-1 ($4.04 \mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$). The results showed that % superiority over check for CRC-9 was 12.48% under

irrigated conditions while it was 16.28% under moisture deficit stress conditions. Whereas for CRC-2 it was 6.15% under irrigated conditions while under stress conditions it was -12%.

With limited available moisture under rain-fed conditions, the crops need to have efficient physiological mechanisms such as ELWRC to rationalize its use. This trait can be used for screening germplasm for drought tolerance in castor and the trait is heritable (Rao *et al.*, 1998) [28]. In blackgram, the genotype with better ELWRC recorded high seed yield (Jyothi Lakshmi *et al.*, 2009) [18] revealing the importance of this trait for selecting drought tolerant genotype. ELWRC was also shown to be associated with some of the key plant characters which have a great bearing on seed yield in rapeseed (Thurling *et al.*, 1980) [34]. In pearl millet, the hybrid ICMH 356 with high seed yield had better ELWRC, hence it was suggested as trait to screen drought tolerance (Vijayalakshmi *et al.*, 2012) [36]. RWC is also an important indicator for plants to withstand drought and used to quantify tolerance to moisture stress (Virginia, 2012) [37]. Decrease in RWC was observed in sorghum hybrid under moisture deficit stress at all growth stages (Yadav *et al.*, 2005) [38]. In the present study, the selected castor genotype CRC-9 was found better for both ELWRC and RWC and is expected to tolerate moisture stress. Both CRIDA castor lines- CRC-2 and CRC-9 recorded higher Pn, gs, Tr and WUE over the check 48-1 under both irrigated and moisture deficit stress conditions. Better WUE was recorded by CRC-9 than 48-1 under both irrigated and moisture deficit stress conditions, whereas CRC-2 fared well than 48-1 only under irrigated condition. Among the selected two genotypes, CRC-9 performed better than CRC-2 and 48-1 for all the physiological traits under both irrigated and drought conditions. Among the genotypes, the Pn of CRC-9 was less affected with moisture deficit stress than CRC-2 and 48-1 and with improved WUE. As this genotype was able to maintain better plant water status and it is evident from its better ELWRC and RWC. High leaf WUE is one of the criteria of drought-tolerance and a water-saving strategy in plant growth (Liu *et al.*, 2012) [22]. Decrease in the photosynthesis under water stress in plants was reported by Liu *et al.*, (2011) [21] as drought decreases stomatal conductance which in turn limit the availability of CO_2 for photosynthesis (Pessarakli, 2001) [26]. Hirayama *et al.*

(2006) [15] suggested that plants exposed to moisture deficit stress, close their stomata to maintain their inner moisture content and consequently their transpiration rate will be decreased. Drought stress causes not only a substantial damage to photosynthetic pigments, but it also leads to deterioration of thylakoid membranes and thus, a reduction in photosynthetic capacity in plants exposed to drought stress is expected (Huseynova *et al.*, 2009, Anjum *et al.*, 2011, Kannan and Kulandaivelu, 2011) [17, 1, 19]. Munoz *et al.* (1998) [25] opined that the genotypes with high yield potential and with high yield under water limited condition are generally associated with reduced WUE mainly because of high water use (Blum, 2005) [6]. Improved WUE on the basis of reduced water use is expressed in improved yield under water-limited conditions only when there is need to balance crop water use against a limited and known soil moisture reserve. However, under most dryland situations where crops depend on unpredictable seasonal rainfall, the maximization of soil moisture use is a crucial component of drought avoidance, which is generally expressed in lower WUE (Blum, 2005) [6].

Water-use efficiency is often considered an important determinant of yield under stress and even as a component of crop drought resistance. It has been used to imply that rain-fed plant production can be increased per unit water used, resulting in more crop per drop (Blum, 2009) [7]. In other drought environments where stress may develop at any time during the season there is no consistent relationship between plant production and WUE (Condon *et al.*, 2002) [29]. On the other hand, the increase in WUE with drought was primarily may be because of stomatal conductance, and thus water loss declined more than carbon fixation (Edwards *et al.*, 2012) [10]. Previous reports have revealed that some genotypes may have more WUE than others, and artificial selection on genotypes with high WUE has been investigated as a means to improve crops with increased yield in water-limited environments (Condon *et al.*, 2002; Rebetzke *et al.*, 2002) [29, 8]. Vijayalakshmi *et al.* (2014) [34] reported that higher WUE in greengram was due to low stomatal conductance and less transpiration.

Table 2 (a): Mean performances of castor genotypes over check for seed weight and physiological components under irrigated and moisture deficit stress conditions

Genotype	Seed yield (g/pl)		Pn ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)		gs (cm s^{-1})		Tr ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)		WUE ($\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$)		Relative water content (RWC)	
	Irr (Rk)	Str (Rk)	Irr (Rk)	Str (Rk)	Irr (Rk)	Str (Rk)	Irr (Rk)	Str (Rk)	Irr (Rk)	Str (Rk)	Irr (Rk)	Str (Rk)
CRC-2	41.45 (1)	27.68 (1)	33.70 (2)	28.77 (2)	0.58 (2)	0.42 (1)	8.67 (2)	8.08 (1)	3.89 (2)	3.56 (3)	73.89 (1)	59.62 (3)
CRC-9	29.18 (3)	22.21 (3)	39.57 (1)	36.90 (1)	0.65 (1)	0.42 (1)	9.61 (1)	7.85 (2)	4.12 (1)	4.70 (1)	69.73 (3)	64.69 (2)
48-1	36.45 (2)	22.57 (3)	29.87 (3)	20.90 (3)	0.50 (3)	0.21 (2)	8.16 (3)	5.17 (3)	3.66 (3)	4.04 (2)	73.53 (2)	69.68 (1)

Irr- irrigated, str-stress, Rk-ranks

Table 2 (b): Percentage superiority (% sup.) over check (48-1) of castor genotypes over check for seed weight and physiological components under irrigated and moisture deficit stress conditions

Genotype	Seed yield (g/pl)		Pn ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)		Gs (cm s^{-1})		Tr ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)		WUE ($\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$)		Relative water content (RWC)	
	Irr % sup.	Str % sup.	Irr % sup.	Str % sup.	Irr % sup.	Str % sup.	Irr % sup.	Str % sup.	Irr % sup.	Str % sup.	Irr % sup.	Str % sup.
CRC-2	13.72	22.64	12.82	37.66	16.00	100	6.25	56.29	6.15	-12.00	0.49	-14.43
CRC-9	-19.95	-1.60	32.47	76.56	30.00	100	17.77	51.84	12.48	16.28	-5.17	-7.16

Irr- irrigated, str-stress

The ANOVA results revealed that seed yield and all the physiological parameters (except WUE) showed significant

variability for the genotypes and also for moisture levels (Table-3).

Table 3: Analysis of variance for physiological parameters in castor genotypes *Significance at $p < 0.05$ and **Significance at $p < 0.01$

Source	DF	Mean sum of squares				
		Seed yield	Pn	gs	Tr	WUE
Replication	2	62.203	4.927	0.009	0.557	0.039
Genotypes	2	118.676**	248.345**	0.053*	7.328**	1.002*
Moisture levels	1	599.118**	137.227**	0.227**	14.187**	0.296
G x ML	1	23.437	15.274	0.006	2.166	0.427
Error	2	12.874	4.851	0.008	0.836	0.192
CV (%)		11.99	6.97	18.63	11.54	10.91

Correlations: The genotypic (r_G) and phenotypic correlations (r_P) for all the physiological parameters and seed yield under both irrigated and moisture deficit stress conditions are presented in Table-4. Seed yield showed significant and positive correlation for Pn, gs and Tr while significantly negatively correlated with WUE under moisture deficit stress conditions. It was observed that Pn is significantly and positively correlated genotypically with gs under both irrigated and moisture deficit conditions. Flexas *et al.* (2002) [13] observed significant and positive correlation of Pn with Tr

in grape wine, gs with Tr in sugarcane (Silva *et al.*, 2013) [33], while in rice significant and positive correlation of Pn with gs (Giuliani *et al.*, 2013) [14] and gs with Tr (Barbour *et al.*, 2010) [3]. The positive correlation between the seed yield and the photosynthesis in wheat has been reported earlier by Makino *et al.* (2011) [24], with gs in maize (Shaibu *et al.*, 2015) [31]. The non-significant correlation between yield and photosynthetic rate was noted in bread wheat (Evans 1993, 1998) [11, 12], with gs in soybean (Liu *et al.*, 2012) [22] with Tr and WUE in Brassicas (Sharma, 2015) [32].

Table 4: Genotypic and phenotypic correlations for physiological parameters of castor genotypes under irrigated and moisture deficit stress conditions

		Seed yield		Pn		gs		Tr		WUE	
		Irri	Stress	Irri	Stress	Irri	Stress	Irri	Stress	Irri	Stress
Pn	r _{IG}	-0.183	0.998**	1.00	1.00	0.998**	0.946*	0.995**	0.875	-0.995**	0.773
	r _{IP}	-0.107	-0.881	1.00	1.00	0.690	0.735	0.974*	0.748	0.679	0.512
gs	r _{IG}	0.102	0.997**			1.00	1.00	0.999**	0.995**	-0.997**	0.444
	r _{IP}	-0.111	-0.781			1.00	1.00	0.744	0.993**	0.731	-0.192
Tr	r _{IG}	-0.174	0.997**					1.00	1.000	-0.986*	0.306
	r _{IP}	-0.229	-0.009					1.00	1.000	-0.609	-0.182
WUE	r _{IG}	-0.233	-0.999**							1.00	1.00
	r _{IP}	-0.460	0.018							1.00	1.00
Seed yield	r _{IG}	1.00	1.00								
	r _{IP}	1.00	1.00								

*Significance at $p < 0.05$ and **Significance at $p < 0.01$

Genotypic and phenotypic variability, heritability and genetic advance as percent of mean (GAM %): The variances, coefficient of variations, heritability and genetic advance as percent of mean (GAM) were presented in Table-5. For all the physiological parameters, the phenotypic variance was higher than the genotypic variance and similarly phenotypic co-efficient of variation (PCV) was also higher than genotypic co-efficient of variation (GCV) indicating the influence of environment in both irrigated and moisture deficit stress conditions. Under irrigated conditions, seed yield and gs showed moderate heritability with moderate

GAM. High heritability with high GAM was found for Pn. Under moisture deficit stress, seed yield showed moderate heritability with low GAM while high heritability with high GAM was found for Pn and Tr. Khatun *et al.* (2015) [20] reported high heritability with high GAM for Pn in upland rice. Hubick *et al.* (1986) [16] reported high heritability for transpiration rate in peanut. Majidi *et al.* (2015) [23] reported moderate heritability for seed yield in Brassica for drought tolerance. Moderate heritability for gs was reported by Basnayake *et al.* (2015) [4] in sugarcane under water-limited conditions.

Table 5: Co-efficient of variation, variances, heritability and genetic advance for physiological parameters of castor genotypes under irrigated and moisture deficit stress conditions

	Genotypic Variance		Phenotypic Variance		GCV		PCV		Heritability		GAM (%)	
	Irri	Stress	Irri	Stress	Irri	Stress	Irri	Stress	Irri	Stress	Irri	Stress
Seed Yield	9.41	2.638	18.94	22.78	8.59	6.72	12.19	19.75	0.497	0.316	12.49	4.71
Pn	23.65	60.68	24.30	70.64	14.14	26.99	14.34	29.12	0.973	0.859	28.75	51.55
Gs	0.004	0.01	0.009	0.020	10.41	30.29	16.75	40.46	0.386	0.560	15.06	45.78
Tr	0.52	2.22	0.57	3.42	8.22	21.19	8.58	26.31	0.916	0.648	16.21	35.15
WUE	0.01	0.30	0.02	0.66	2.66	13.29	3.81	19.58	0.485	0.461	3.93	18.80

Conclusion: CRIDA castor genotype CRC-9 performed better than the check for the four physiological traits studied viz., ELWRC, Pn, gs and WUE under both irrigated and moisture deficit stress conditions. However, CRC-2 performed better than the check for two physiological traits viz., gs and Tr under moisture deficit conditions only. Pn, gs and Tr registered high heritability with high GAM under moisture deficit stress conditions and can be considered as important physiological traits for the crop improvement of castor under drought conditions.

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