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Three-Way Top-Cross Hybrids to Enhance Production of Forage with Improved Quality in Pearl Millet (*Pennisetum glaucum* (L.) R. Br.)

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Abstract: Three-way top-cross hybrids of pearl millet were evaluated along with a popular single-cross check hybrid (PAC 981) for forage yield and quality traits under a multi-cut (three cuts) system across multiple years, seasons and sites in India. Total green forage yield (TGFY) varied from 36 to 53 t ha^{-1}, and two hybrids outyielded the check hybrid for both total dry forage yield (TDFY) and forage quality (CP; Crude protein, and IVOMD; In vitro organic matter digestibility) traits. A set of promising three-way top-cross hybrids evaluated along with a set of promising open-pollinated varieties (OPVs) and top-cross hybrids for forage-related traits over two years under a multi-cut system revealed that the mean TDFY of three-way top-cross hybrids was higher than the mean TDFY of top-cross hybrids, followed by OPVs. Also, three-way top-cross hybrids had higher/or at par forage quality traits such as CP and IVOMD in comparison to other types of cultivars. TDFY had no correlation with CP and IVOMD across cuts in three-way top-cross hybrids, indicating that forage quantity and quality traits can be improved independently of each other. Overall, three-way top-cross hybrids were found to be a better pearl millet cultivar option than other types of cultivars.

Keywords: open-pollinated varieties; top-cross hybrids; three-way top-cross hybrids; forage yield; forage quality

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1. Introduction

Pearl millet (*Pennisetum glaucum* (L.) R.Br) is an important climate-resilient cereal food crop that is grown on more than 30 m ha around the globe, especially in the hot and dry areas of Africa and Asia. This crop has high dry matter per day productivity due to a C_4 photosynthetic pathway, is a warm season annual and has high tillering with tolerance to drought, salinity and low soil fertility stresses. It has an inherent ability to grow well in harsher climates that other cereal crops such as rice, wheat and sorghum cannot tolerate [1]. This crop is primarily cultivated for grain, but in some parts of the world it is grown exclusively for forage. For instance, pearl millet is grown as summer pasture in the southern USA [2,3], in some parts of northwestern India during the summer season [4,5], in Brazil [6,7] and in some central Asian countries [8].

At present, India is the largest producer of milk in the world and is projected to produce 400 million tons by 2050 [9]. Lives of livestock and smallholder farmers in the marginal environments are affected due to the occurrence of droughts, floods, pests and diseases and poor soil fertility. Such conditions lead to drastic reduction of the productivity of milching animals due to scarcity of fodder and feed. For example, at present, India faces a net deficit of 590 million tons of green fodder and 468 million tons of dry fodder, and it would require around 1013 million tons of green fodder and 631 million tons of dry fodder by 2050 [10]. To fulfil these requirements, the pearl millet crop might be the farmer's choice, as it has a high forage yielding potential, wider adaptation, rapid regrowth and absence of any anti-nutritional factors such as hydrocyanic and prussic acid. As such, it offers multiple harvests to ensure the regular supply of forages [11].

Efforts are underway to ensure forage productivity through the use of new breeding materials, open-pollinated varieties (OPVs) and single/top-cross hybrids in pearl millet under a single-/multiple-cut system [12–17]. Nonetheless, smallholder dairy farmers are now demanding multi-cut forage cultivars to increase forage production from the same area of land. Few studies have been conducted on the multi-cut system in pearl millet for forage yield and quality traits [18–21]. The higher seeding rate required in forage crops is another challenge that increases the seed cost for the farmers. Under such circumstances, a three-way top-cross hybrid methodology (involvement of three diverse parents: two inbreds and one OPV) has the potential to economize the seed production cost and also might be a good option to improve the forage production efficiency of fields.

Studies conducted to compare the grain yield potential of sterile F_1 hybrids (derived from crosses between A-lines and non-isogenic B-lines) and their inbred seed parents in pearl millet found that the F_1 sterile hybrids (female parent of three-way hybrid) produce 64 to 107% higher seed yield than their higher-yield inbred seed parents [22–25]. Few studies have also demonstrated that seed yield of three-way cross hybrids (involvement of three diverse inbred parents) is double as compared to single-cross hybrids in maize [26] and in sunflower [27].

Relatively few studies have been reported on the comparative performance of different type of cultivars for forage-related traits in pearl millet. For instance, it was found that single-cross and three-way cross hybrids had almost the same forage yields [23]. Also, a study conducted on the multi-cut system in pearl millet concluded that top-cross hybrids outyielded OPVs at the first cut, but the forage yields of top-cross hybrids and OPVs were at par at the second cut [18]. Another study conducted by Gupta et al. [16] showed that promising OPVs (17 t ha^{-1}) were higher than top-cross hybrids (14.3 t ha^{-1}) for dry forage yield at 85-90 days in a single cut after planting pearl millet.

The present study aimed at assessing the forage production potential of the three-way top-cross hybrids in comparison to OPVs and top-cross hybrids.

2. Materials and Methods

2.1. Plant Materials

Ten sterile hybrids (A × B) were developed using four A- lines of A_5 cms (Cytoplasmic male sterility) crossed to ten different B- lines (A_5 cms maintainer) in different combinations. Each one of these ten combinations had A- and B- lines from different genetic backgrounds. These ten F_1s (A × B sterile hybrids) were crossed with seven open-pollinated varieties (OPVs) as pollinators to produce 70 three-way top-cross hybrids in a line × tester mating design during the summer season (February to May) of 2015. The OPVs used as pollinators in this study were earlier identified as promising for high biomass traits [16].

2.2. Field Evaluations

2.2.1. Experiment 1: Trials to Evaluate Three-Way Top-Cross Hybrids

A trial comprised of 70 three-way top-cross hybrids along with one commercial popular check hybrid PAC 981 (bred by Advanta Seed Ltd., Hyderabad, India) was evaluated for forage yield and quality traits during the rainy season (July to October) of 2015 at a seed company experimental farm near Hyderabad. The check hybrid PAC 981 (Nutrifeed) is a popular multi-cut, high-biomass-yielding, single-cross pearl millet hybrid, and it has occupied a significant area during the summer and rainy seasons in India for the last 10 years. This trial was evaluated in a randomized complete block design with two replications, each entry was planted in 4 rows of 4 m length and rows were spaced 50 cm apart. Based on green forage yield (GFY: 34 to 63 t ha^{-1}) and quality traits (CP: 8 to 12% and IVOMD: 42 to 50%) data, 29 promising three-way top-cross hybrids were identified (data not provided). These identified hybrids had higher or on par forage yield and forage quality traits in comparison to the check hybrid PAC 981.

These identified twenty-nine three-way top-cross hybrids along with check PAC 981 were evaluated under three environments: the rainy season of 2017, the summer season of 2018 at PAU, Ludhiana, India (30° N, 75° E and 247 m above sea level) and the summer season of 2018 at ICRISAT, Patancheru, India (18° N, 78° E and 545 m above sea level). The rainfall and temperature data for the sites during the experimental period are provided in Table 1.

Table 1. Characteristics of weather parameters at the experimental sites.

Experiment	Location	Year	Crop Season	Overall Rainfall (mm)	Temperature (°C) (Mean Values over the Crop Season)	
					Maximum	Minimum
Experiment 1	PAU, Ludhiana	2017	Rainy (July to October)	177.8	38.0	26.5
Experiment 1	PAU, Ludhiana	2018	Summer (February to May)	122.6	34.8	21.0
Experiment 1	ICRISAT, Patancheru	2018	Summer (February to May)	49.4	36.1	19.8
Experiment 2 (Trial 1)	ICRISAT, Patancheru	2018	Rainy (July to October)	336.2	30.8	21.6
Experiment 2 (Trial 2)	ICRISAT, Patancheru	2019	Rainy (July to October)	679.4	30.4	21.8

2.2.2. Experiment 2: Comparison of Three-Way Top-Cross Hybrids, OPVs and Top-Cross Hybrids

One hundred and nineteen top-cross hybrids (derived by crossing seed parents and OPVs as pollinators) evaluated in various breeding trials during 2014–2017 at ICRISAT led to the identification of 18 superior top-cross hybrids. Similarly, 52 OPVs developed as promising forage cultivars evaluated in multilocation and multiyear trials during 2015-2017 at ICRISAT led to the identification of 25 superior OPVs. These identified top-cross hybrids, OPVs and the three-way top-cross hybrids identified from experiment 1 were evaluated in the two multi-cut forage pearl millet trials conducted over two years (2018 and 2019) in the rainy season at ICRISAT, Patancheru, India. The number of cultivars evaluated in multi-cut forage pearl millet trial in both the years was different, as poor performing cultivars were discarded and new promising cultivars were added in subsequent trialing. Both of the trials included the hybrid PAC 981 as a check hybrid. The details of the trials are as follows: Trial 1: During the rainy season of 2018, the trial comprising of 25 OPVs, 18 top-cross hybrids and the best 10 three-way top-cross hybrids (identified from Experiment 1) were evaluated for forage quantity and quality traits at ICRISAT, Patancheru, India. Trial 2: During the rainy season of 2019, 20 OPVs, the 5 top-cross hybrids and 15 three-way top-cross hybrids were evaluated for forage traits at ICRISAT, Patancheru, India. Of these two trials conducted over 2 years, 12 OPVs, 4 top-cross hybrids and 7 three-way top-cross hybrids commonly found were compared further for forage yield and quality traits.

All of the trials in this experiment were evaluated in a randomized complete block design with two replications. Each entry was planted in 4 rows of 4 m length, except at PAU, Ludhiana, India where 6 rows of 4 m length were planted. Rows were spaced 30 cm apart during the summer season of 2018 at PAU, Ludhiana, India, whereas rows were spaced 60 cm apart in the summer season of 2018, and 75 cm in the rainy seasons of 2018 and 2019 at ICRISAT, Patancheru. At PAU, Ludhiana, India, the experimental area was fertilized with 50 kg N ha^{-1} and 60 kg P ha^{-1} at the time of crop establishment. The entire experimental plot was top-dressed thrice with the rate of 25 kg N ha^{-1} when plants were about knee-high (30 days after planting) before the first harvest, immediately after the first cut at 50 days after planting and after the second harvest (30 days after first cut) at PAU, Ludhiana, India. At ICRISAT, Patancheru, India, 18 kg N ha^{-1} and 46 kg P ha^{-1} of Diammonium phosphate were applied at the time of field preparation, and the field was fertilized thrice with a dosage rate of 100 kg ha^{-1} of urea (46% N) as top-dressing (30 days after planting), immediately after the first cut (50 days after planting) and after the second cut (30 days after first cut) at ICRISAT, Patancheru, India. Trials were irrigated at a 12- to 15- day interval, and the crop was protected from diseases and pests during the whole cropping period.

2.3. Estimation of Forage Traits

2.3.1. Biomass Related Traits

Three forage-cutting intervals were followed in Experiment 1 across locations and also in Experiment 2 (Trial 2), conducted during the rainy season of 2019. The following schedule of cuts was followed: first cut (50 days after sowing), second cut (30 days after first cut) and third cut (30 days after second cut). By contrast, only two cuts (first and second cuts, respectively) were taken in Experiment 2 (Trial 1), conducted during the rainy season of 2018 at ICRISAT, Patancheru. A plot of four rows of each entry was harvested manually at 10 to 12 cm height (leaving at least 2 nodes) from the ground level at the first cut. At the time of harvest (at each cut), plant height (PH, cm) was measured on 5 random plants from the base of the stem to the tip of a panicle of the main tiller. The fresh weight of the green forage was recorded (kg) on a plot basis (PAU: Summer (7.2 m^2) and Rainy (4.8 m^2); ICRISAT: Summer (9 m^2) and Rainy (12 m^2)). A subsample (10–15 plants) of about 1 kg was collected per entry at the time of harvest and recorded for green forage weight, oven dried for 8 h daily for three to four days at 60 °C in a Campbell dryer (Campbell Industries, Inc., 3201 Dean Avenue, Des Moines, IA, USA) and reweighed (dry forage weight in kg). The dry matter (DM) concentration was determined by the ratio between the dry forage weight and the green forage weight, and also the dry forage yield (DFY) of each entry was calculated by multiplying the green forage weight and the dry matter concentration. The green forage yield (GFY) and DFY were converted into t ha^{-1}. The second (at 80 days) and third (at 110 days) cuts of forage were harvested from the same plot of four rows after 30 days after the first and second cuts, respectively. The PH, GFY, DFY and forage quality traits were also recorded in the second and third cuts, as described in the first cut. The total green forage yield (TGFY) and total dry forage yield (TDFY) in t ha^{-1} were calculated as the sum of all the cuts for each entry in these trials.

2.3.2. Forage Quality Traits

Forage quality traits were analysed for entries planted in trial only at the ICRISAT, Patancheru location in both of the experiments, as it was not possible to procure forage samples from other locations. The dried subsamples of the whole plant (10–15 plants) of each entry were chopped into 10 to 15 mm pieces using a chaff cutter (Model # 230, Jyoti Ltd., Vadodara, India) and ground in a Thomas Wiley mill (Model # 4, Philadephia, PA, USA) through a 1-mm screen for chemical analysis. Ground stover samples (approximately 40 g of sample/entry) were analyzed by Near-Infrared Reflectance Spectroscopy (NIRS) for stover nitrogen concentration (N%), crude protein (calculated using N% × 6.25) and IVOMD, as described by Bidinger and Blummel [28] and Blummel et al. [14].

2.4. Data Analysis

Data collected from the three cuts for forage-related traits in Experiment 1 and 2 were subjected to analysis of variance (ANOVA) using GenStat® 18th edition (VSN International Limited, Hemel Hempstead, UK). The mean data of forage-related traits were used for simple Pearson's correlation coefficients using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA, USA).

3. Results and Discussion

3.1. ANOVA of Forage-Related Traits for Multilocation Trial (Experiment 1)

The combined ANOVA indicated significant differences among genotypes for PH, GFY, DFY, TGFY and TDFY across all cuts and CP and IVOMD for the third cut (except CP and IVOMD at the first and second cuts, respectively) (Table 2). Environment and genotype × environment interactions were significant for all the traits, indicating hybrids significantly varied at different locations for forage-related traits. This result was in accordance with those of earlier reported studies in pearl millet hybrid parents/single-cross hybrids for GFY and DFY [17,29].

The mean performance of three-way top-cross hybrids over three locations are presented in Table 3. The TGFY and TDFY among hybrids varied from 36 to 53 t ha^{-1} and 7 to 12 t ha^{-1}, respectively. Forage yields reported in the current study are comparable with forage yields reported by earlier studies in pearl millet [16–19]. The check hybrid PAC 981 had 42 t ha^{-1} TGFY and 9.6 t ha^{-1} TDFY, respectively. Ten and three hybrids were superior to the best check hybrid PAC 981 by ≥15% for TGFY and TDFY, respectively. Among these hybrids, three were found superior for both TGFY and TDFY, respectively, over the best check hybrid PAC 981.

One unit increase in IVOMD in stover sorghum and pearl millet can result in an increase in livestock productivity of 6 to 8% [30]. Across cuts, CP and IVOMD in these experiments varied from 5.8 to 16% and 48 to 62%, respectively, indicating the existence of large variability among the hybrids studied in comparison to the earlier studies [12–16,18]. Most hybrids exceeded the minimum requirement (7%) of CP for rumen microbes [31]. The check hybrid PAC 981 had 10%, 7% and 13% of CP and 57%, 49% and 59% IVOMD at the first, second and third cuts, respectively. Among 29 three-way top-cross hybrids, none, 15 and 4 were found to be superior to the check hybrid PAC 981 for CP at the first, second and third cuts, respectively. In addition, 3, 27 and 2 three-way top-cross hybrids performed better than the check hybrid PAC 981 for IVOMD at the first, second and third cuts, respectively. Two of the three-way top-cross hybrids were identified as superior for both forage yield (≥15% TGFY and TDFY) and for important forage quality traits (27% and 11% CP and 6% and 4% IVOMD at the second cut, and comparable percentages for the first and third cuts) over PAC 981.

Table 2. Combined analysis of variance for forage traits of 29 three-way top-cross hybrids along with check hybrid in pearl millet, evaluated at PAU, Ludhiana in rainy 2017 and summer 2018; and at ICRISAT, Patancheru in summer 2018.

Source of Variation	d.f.	Forage Quantity Related Traits (Three Environments)											Forage Quality Traits (Only for ICRISAT Location)					
		PH			GFY			DFY			TGFY	TDFY	CP			IVOMD		
		FC ‡	SC †	TC ǂ	FC	SC	TC	FC	SC	TC	All Cuts	All Cuts	FC	SC	TC	FC	SC	TC
Environment	2	241405 ***	119974.3 ***	106008.75 ***	6874.73 ***	220.85 *	722.32 ***	411.50 ***	4.7224	39.77 ***	2030.56 ***	350.86 ***	NA	NA	NA	NA	NA	NA
Replication (Env.)	3 (1) Ī	287.90	261	775.04 **	24.84	18.372	1.4673	2.20	1.431	0.31 **	66.6	1.25	0.5	9.57 **	17.46 *	22.82 **	0.43	9.90 *
Genotype	29	554 ***	475.4 ***	398.54 ***	47.5 **	36.79 ***	2.99 ***	4.21 ***	1.82 ***	0.17 ***	132.2 ***	8.27 *	1.53	0.87	5.44 *	2.29	5.56	6.49 **
Genotype × Environment	58	359.9 ***	462.3 ***	214.91 ***	49.24 ***	21.66 ***	2.99 ***	3.26 ***	2.10 ***	0.16 ***	104.52 ***	9.07 ***	NA	NA	NA	NA	NA	NA
Error	87 (29)	118	126	92.41	24.7	7.36	0.89	1.83	0.80	0.48	56.37	4.98	0.61	0.66	2.49	2.26	6.99	2.01

Note: df-Degrees of freedom, PH (cm)-Plant height, GFY (t ha^{-1})-Green forage yield, DFY (t ha^{-1})-Dry forage yield, TGFY (t ha^{-1})-Total green forage yield, TDFY (t ha^{-1})-Total dry forage yield, CP (%)-Crude protein and IVOMD (%)-In vitro organic matter digestibility. *, ** and *** indicated significant at 0.05, 0.01 and 0.001 level, respectively. FC ‡-First cut, SC †-Second cut and TC ǂ-Third cut. NA-Not available. Ī Values mentioned in the parenthesis are degrees of freedom for forage quality traits.

Table 3. Mean performances of 29 three–way top-cross hybrids along with the check for forage related traits in pearl millet, evaluated at PAU, Ludhiana in rainy 2017 and summer 2018, and at ICRISAT, Patancheru in summer 2018.

S. No.	Entry	Forage Quantity Related Traits (Three Environments)												Forage Quality Traits (at ICRISAT)					
		First Cut			Second Cut			Third Cut			Combined of All Three Cuts		% Over PAC 981 for TDFY	First Cut		Second Cut		Third Cut	
		PH (cm)	GFY (t ha^{-1})	DFY (t ha^{-1})	PH (cm)	GFY (t ha^{-1})	DFY (t ha^{-1})	PH (cm)	GFY (t ha^{-1})	DFY (t ha^{-1})	TGFY (t ha^{-1})	TDFY (t ha^{-1})		CP (%)	IVOMD (%)	CP (%)	IVOMD (%)	CP (%)	IVOMD (%)
1	†TWTCH 01	119.0	19.9	3.7	146.0	21.1	4.9	104.0	6.8	1.4	47.8	10.0	4.2	8.3	54.6	7.1	50.3	12.9	58.4
2	TWTCH 02	128.0	25.2	5.7	163.0	18.3	4.2	103.0	5.7	1.1	49.2	11.0	14.6	7.1	55.2	5.9	48.9	11.2	54.3
3	TWTCH 03	131.0	19.8	4.8	159.0	18.5	4.3	92.0	5.2	1.2	43.5	10.3	7.3	7.1	54.0	5.8	54.0	10.0	56.0
4	TWTCH 04	118.0	17.7	3.4	153.0	17.5	3.7	82.0	5.3	1.0	40.4	8.1	−15.6	7.8	55.6	6.8	51.1	10.5	56.4
5	TWTCH 05	110.0	21.3	5.9	140.0	22.2	4.9	82.0	5.2	1.2	48.7	12.0	25.0	8.3	55.5	8.5	51.4	9.6	56.5
6	TWTCH 06	113.0	19.1	3.7	150.0	16.5	3.7	87.0	5.1	1.0	40.7	8.5	−11.5	8.5	56.3	8.6	51.8	12.6	56.9
7	TWTCH 07	115.0	18.6	3.8	136.0	17.4	4.5	89.0	6.3	1.2	42.3	9.5	−1.0	6.5	55.7	6.4	49.3	9.9	54.1
8	TWTCH 08	118.0	22.5	5.5	149.0	21.1	4.0	94.0	6.0	1.1	49.7	10.6	10.4	7.4	53.2	6.8	51.1	12.9	58.1
9	TWTCH 09	129.0	21.5	4.5	154.0	20.6	5.0	99.0	6.8	1.1	49.0	10.6	10.4	7.6	57.7	7.4	52.9	11.1	56.5
10	TWTCH 10	134.0	20.8	4.8	162.0	23.5	5.4	99.0	6.4	1.2	50.8	11.5	19.8	5.8	54.4	7.4	50.6	11.5	56.6
11	TWTCH 11	119.0	20.2	4.6	168.0	21.2	4.8	106.0	6.9	1.3	48.3	10.7	11.5	7.7	55.1	7.0	51.4	10.6	55.9
12	TWTCH 12	127.0	20.6	4.3	157.0	23.0	5.3	92.0	6.6	1.1	50.2	10.7	11.5	7.5	55.1	7.2	48.2	11.8	54.6
13	TWTCH 13	120.0	22.5	4.8	153.0	24.1	4.4	110.0	6.2	1.0	52.8	10.1	5.2	8.9	56.7	7.6	50.6	14.2	57.4
14	TWTCH 14	110.0	20.8	3.7	150.0	19.4	3.7	101.0	6.0	1.1	46.3	8.5	−11.5	7.2	55.1	6.2	52.2	10.6	57.3
15	TWTCH 15	111.0	19.8	4.3	142.0	18.7	4.7	96.0	5.5	1.0	44.0	10.0	4.2	8.5	56.0	6.4	51.6	12.5	57.8
16	TWTCH 16	123.0	18.1	4.4	142.0	22.3	4.7	96.0	4.6	0.9	45.0	10.0	4.2	8.5	55.6	6.5	49.8	11.6	58.1
17	TWTCH 17	120.0	17.5	3.9	144.0	19.4	4.0	81.0	4.7	0.7	41.6	8.7	−9.4	8.8	56.3	6.2	52.4	12.4	56.7
18	TWTCH 18	115.0	15.7	3.3	143.0	15.2	3.9	83.0	4.6	0.9	35.6	8.2	−14.6	6.9	54.8	7.2	53.1	10.3	56.0
19	TWTCH 19	134.0	13.7	2.5	161.0	19.2	4.7	86.0	5.6	1.3	38.5	8.5	−11.5	8.0	56.6	6.5	50.8	10.5	53.7
20	TWTCH 20	129.0	15.4	3.0	145.0	18.1	3.8	110.0	5.0	0.9	38.6	7.7	−19.8	7.8	55.0	6.3	49.8	8.5	55.1
21	TWTCH 21	114.0	15.7	3.6	154.0	15.7	3.7	97.0	5.3	1.0	36.6	8.3	−13.5	7.8	54.6	6.8	51.8	10.3	56.3
22	TWTCH 22	134.0	14.5	3.1	160.0	16.2	3.2	94.0	5.4	1.0	36.1	7.3	−24.0	7.5	57.2	6.5	50.3	10.2	54.9
23	TWTCH 23	126.0	16.8	3.5	156.0	19.7	4.1	89.0	5.5	1.0	42.0	8.7	−9.4	7.2	56.7	6.5	53.6	11.9	55.4
24	TWTCH 24	128.0	15.7	3.8	157.0	16.6	3.6	99.0	6.1	1.4	38.5	8.7	−9.4	7.2	54.8	6.8	49.8	13.2	58.0
25	TWTCH 25	110.0	15.7	3.1	155.0	16.3	3.8	88.0	4.7	0.9	36.7	7.9	−17.7	7.5	54.2	6.7	52.6	14.5	59.4

26	TWTCH 26	119.0	15.6	3.6	149.0	15.5	3.7	98.0	5.8	1.2	36.9	8.4	−12.5	8.2	55.9	7.0	54.5	12.2	54.5
27	TWTCH 27	116.0	21.7	4.9	143.0	17.0	4.3	107.0	6.3	1.2	44.9	10.4	8.3	8.4	57.2	5.8	48.5	9.9	56.7
28	TWTCH 28	134.0	21.8	5.0	154.0	17.6	4.0	90.0	5.3	1.0	44.8	10.1	5.2	7.3	54.6	6.3	52.9	9.9	55.2
29	TWTCH 29	109.0	18.2	4.5	151.0	19.3	3.6	92.0	5.9	0.9	43.4	9.1	−5.2	8.9	55.7	6.8	50.0	15.8	62.1
30	Check (PAC 981)	93.0	19.5	4.6	127.0	17.9	4.3	93.0	4.3	0.7	41.7	9.6		10.5	56.9	6.7	48.6	13.2	59.0
Grand mean		120.0	18.9	4.1	151.0	19.0	4.2	10.1	5.6	1.1	42.5	12		7.8	55.5	6.8	51.1	11.5	56.6
Coefficient of variation (%)		9.0	26.3	32.6	7.4	14.3	21.2	9.6	16.7	20.5	17.7	25.4		11.5	2.7	12.0	5.2	13.7	2.5
Standard error		10.9	5.0	1.4	11.2	2.7	0.9	9.6	0.9	0.2	7.5	2.2		0.9	1.5	0.8	2.6	1.6	1.4

Note: † TWTCH: Three-way top-cross hybrids**.** PH−Plant height, GFY−Green forage yield, DFY−Dry forage yield, TGFY−Total green forage yield, TDFY−Total dry forage yield, CP−Crude protein and IVOMD−In vitro organic matter digestibility.

3.2. Correlations among Forage Yield and Quality Traits

The TDFY was found significantly positively correlated with TGFY (r = 0.86, $p < 0.0001$) (Figure 1a), and also with GFY (r = 0.78, $p < 0.0001$ at first cut and r = 0.52, $p < 0.01$ at second cut) and DFY (r = 0.83, $p < 0.0001$ and r = 0.65, $p < 0.0005$, for first and second cuts, respectively) (data not shown). Similar correlations between forage quantity traits were also reported earlier by Imran et al. [32] and Govintharaj et al. [33] in pearl millet. Similarly, for the forage quality trait, IVOMD had a significant positive correlation with CP (r = 0.41, $p < 0.05$ and r = 0.72, $p < 0.0001$ for the first and third cuts, respectively) (data not shown). The TDFY had no correlation with CP and IVOMD across cuts (Figure 1b,c), indicating that forage quantity and quality traits can be improved independently. Such results have also been earlier reported in sorghum [34].

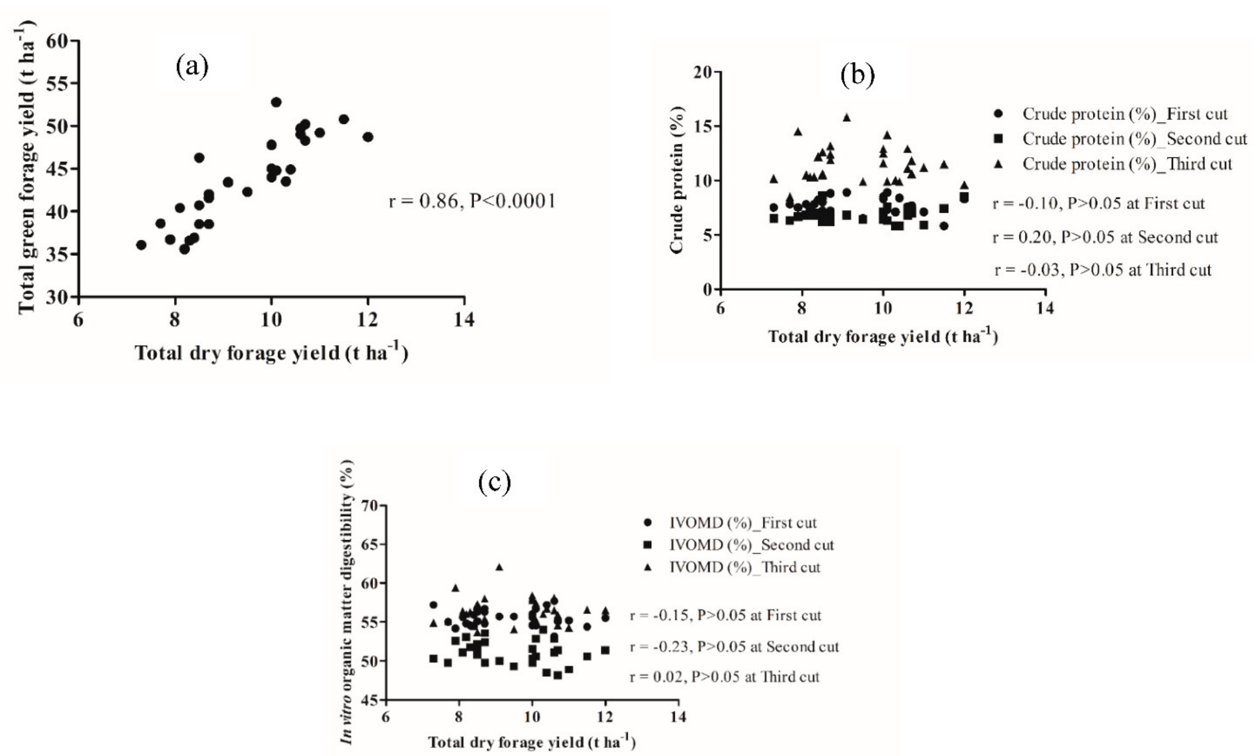

Figure 1. Correlations between (**a**) Total green forage yield (TGFY) and total dry forage yield (TDFY); (**b**) Total dry forage yield (TDFY) and crude protein (CP) and (**c**) Total dry forage yield (TDFY) and in vitro organic matter digestibility (IVOMD) in 29 three-way top-cross hybrids in pearl millet.

3.3. Performances of Different Forage Type Cultivars: OPVs, Top-Cross Hybrids and Three-Way Top-Cross Hybrids (Experiment 2)

Overall mean performance of the promising 23 (12 OPVs, 4 top-cross and 7 three-way top-cross hybrids) cultivars for forage related traits is shown in Table 4. Three-way top-cross and top-cross hybrids matured six to thirteen days earlier than OPVs, and they yielded significantly higher green forage, suggesting that hybrid cultivars can save a minimum of about two irrigations, labour and field management costs in comparison to OPVs. These results are in agreement with the results of Rai et al. [18], who found top-cross hybrids were relatively early maturing and produced higher biomass at the 50-day harvest as compared to OPVs in pearl millet.

The TDFY ranged from 5 to 9 t ha^{-1} in OPVs, 8 to 9 t ha^{-1} in top-cross hybrids and 8 to 10 t ha^{-1} in three-way top-cross hybrids. Also, the highest yielding three-way top-cross hybrid, OPV and top-cross hybrid had 43, 37 and 33% higher TDFY than the check hybrid PAC 981, respectively. Furthermore, the four best three-way top-cross hybrids when compared with the best four of each the OPVs and top-cross hybrids for mean TDFY revealed that the three-way top-cross hybrids had higher TDFY than other cultivars (Table 5a). Similarly, the average TDFY of the seven best three-way top-cross hybrids had higher-than-average TDFY than the seven best OPVs (Table 5b). Furthermore, four OPVs, two top-cross hybrids and five three-way top-cross hybrids significantly outyielded the check hybrid PAC 981 by ≥20% of TDFY.

Forage quality traits CP and IVOMD varied from 8 to 13% and 49 to 55% in OPVs, 8 to 13% and 49 to 56% in top-cross hybrids and 9 to 12% and 51 to 54% in three-way top-cross hybrids, respectively, across cuts. The reported mean values of CP and IVOMD in this study were found to be higher than those in the earlier studies in pearl millet [13,14]. Three-way top-cross hybrids had superior forage quality at any of the two cuts (out of 3 cuts) than other cultivars when compared with the four best cultivars among OPVs, top-cross hybrids and three-way top-cross hybrids. One each of OPV and three-way top-cross hybrids had a high forage yield combined with better forage quality traits across cuts over the best check hybrid PAC 981. None of the top-cross hybrid was found superior for both forage yield and quality traits across cuts over the check hybrid PAC 981.

In the present study, the mean values of all of the three-way top-cross hybrids for TDFY were slightly higher than those of the OPVs and top-cross hybrids, whereas forage quality traits were comparable with those of the OPVs (except IVOMD at third cut) and slightly higher than those of the top-cross hybrids (except CP at third cut). However, when we compared the performance of the four best three-way top-cross hybrids and/or individually with other cultivars, we found three-way top-cross hybrids to be better in forage yield and quality traits than OPVs and top-cross hybrids. It was observed that the F_1 sterile hybrids (female parent of the three-way top-cross hybrid) produce 40 to 70% higher seed yield than the female inbred parent of a single-cross-forage hybrid (Dr Aditya Sharma, Advanta India, Personal communication, 2022). Also, three-way top-cross hybrids have an advantage in maintaining the confidentiality (that allows seed companies to commercialize them with confidence) of parental lines (F_1 sterile used as a seed parent (female parent)) in seed production plots, whereas parental lines of single-cross hybrids can be infiltrated by competitors from seed production fields, as is routinely found in the case of single-cross hybrids. Considering the various factors—such as higher forage yield and better/comparable quality traits in three-way top-cross hybrids, their better performances than the other type of cultivars (OPVs and top-cross hybrids) for forage traits, their higher seed yielding potential due to male sterile F_1 as a female parent, the better opportunity to combine traits in a single cultivar due to involvement of three diverse parents, and finally their protection from infiltration—we conclude that three-way top-cross hybrids seem to be the most preferable cultivar for smallholder farmers and seed companies.

Table 4. Mean performances of forage yield and quality traits for OPVs, top-cross and three-way top-cross hybrids, evaluated at ICRISAT in rainy seasons of 2018 and 2019.

S. No.	Entry	Days to 50% Bloom	Forage Quantity Related Traits												Forage Quality Traits					
			Rainy 2018				Rainy 2019					Across Years			CP (%)			IVOMD (%)		
			PH (cm)	PH (cm)	TGFY (t/ha)	TDFY (t/ha)	PH (cm)	PH (cm)	PH (cm)	TGFY (t/ha)	TDFY (t/ha)	TGFY (t/ha)	TDFY (t/ha)	% Over PAC 981 for TDFY	Across Years		Rainy 2019	Across Years		Rainy 2019
			First Cut	Second Cut	All Two Cuts	All Two Cuts	First Cut	Second Cut	Third Cut	All Three Cuts	All Three Cuts				First Cut	Second Cut	Third Cut	First Cut	Second Cut	Third Cut
Open-pollinated varieties																				
1	‡ICMV 05222	89	111.9	226.8	33.7	6.9	190.5	136.0	129.0	55.5	3.0	44.6	4.9	−26.9	11.7	11.0	8.8	51.1	54.0	49.2
2	ICMV 05555	68	117.8	224.5	35.6	8.2	219.0	147.0	124.0	53.6	8.0	44.6	8.1	20.9	10.0	10.3	9.5	51.7	52.6	51.4
3	ICMV 05777	88	142.2	247.0	42.3	8.1	203.0	143.0	132.0	66.1	6.6	54.2	7.3	9.0	10.8	9.7	8.9	51.0	53.3	49.3
4	ICMV 15111	58	199.0	186.5	48.6	5.4	249.5	155.0	120.5	38.2	5.4	43.4	5.4	−19.4	9.6	10.4	11.3	52.4	53.7	55.0
5	ICMV 1602	77	153.5	235.0	37.8	8.0	213.0	146.5	123.0	33.3	7.5	35.6	7.7	14.9	10.4	11.1	9.4	51.6	52.2	50.9
6	ICMV 1605	82	144.0	218.0	39.3	8.8	206.0	138.0	135.0	47.5	6.5	43.4	7.7	14.9	10.1	11.4	9.9	51.3	53.8	49.9
7	ICMV 1608	79	140.5	248.0	45.5	9.5	215.0	137.0	129.5	62.6	6.0	54.1	7.7	14.9	11.2	11.1	9.6	51.7	54.9	53.5
8	ICMV 1613	88	145.0	224.5	40.5	8.9	196.0	156.0	120.0	51.6	5.6	46.1	7.3	9.0	11.5	9.8	8.0	50.7	54.8	49.7
9	ICMV 1617	70	146.0	233.8	38.2	10.0	214.0	150.0	139.5	56.3	6.7	47.2	8.3	23.9	10.1	10.4	12.5	51.6	54.2	54.9
10	ICMV 1701	74	134.0	237.5	37.8	9.6	224.0	151.5	134.5	57.8	6.9	47.8	8.3	23.9	11.4	10.8	11.2	51.0	53.0	50.6
11	ICMV 1707	69	145.0	251.0	43.2	8.8	245.0	142.0	126.0	68.0	7.0	55.6	7.9	17.9	11.7	9.7	8.5	52.1	54.0	49.8
12	ICMV 1708	87	149.5	260.0	45.7	12.2	207.0	174.0	128.5	53.6	6.2	49.6	9.2	37.3	11.4	9.1	9.1	51.7	51.9	50.1
	Mean	77	144.0	232.7	40.7	8.7	215.2	148	128.5	53.7	6.3	47.2	7.5		11.0	10.4	9.8	51.5	53.5	51.2
Top-cross hybrids																				
13	ǂTCH 01	89	216.0	225.0	44.5	10.7	245.0	167.0	122.0	67.0	7.2	55.7	8.9	32.8	9.6	9.7	8.9	50.7	51.1	50.3
14	TCH 02	47	219.0	189.5	45.3	11.7	227.0	162.5	126.0	51.2	6.1	48.3	8.9	32.8	9.1	10.0	12.6	51.2	52.0	55.6
15	TCH 03	77	191.0	252.0	43.8	10.2	238.0	161.0	125.5	54.0	5.6	48.9	7.9	17.9	10.4	9.7	8.3	50.9	52.2	49.5
16	TCH 04	71	200.5	223.0	48.3	10.1	244.5	176.5	136.0	64.4	5.0	56.3	7.6	13.4	8.1	9.5	10.3	49.1	52.3	52.2
	Mean	71	206.6	222.4	45.5	10.7	238.6	166.8	127.4	59.2	6.0	52.3	8.3		9.3	9.7	10.0	50.5	51.9	51.9
Three−way top-cross hybrids																				
17	† TWTCH 01	63	176.5	209.0	41.8	10.1	255.5	147.0	128.5	61.8	5.5	51.8	7.8	16.4	10.2	11.7	11.4	52.7	53.6	53.1

18	TWTCH 02	63	179.5	227.5	38.0	8.0	259.5	164.5	128.0	47.9	9.6	43.0	8.8	31.3	10.7	10.2	10.6	50.7	53.6	53.6
19	TWTCH 03	64	214.0	220.5	46.0	8.5	253.0	166.0	132.0	55.6	10.6	50.8	9.5	41.8	11.3	10.2	9.4	50.8	54.3	53.3
20	TWTCH 04	64	181.0	252.5	42.8	9.6	236.0	158.0	143.0	64.5	6.2	53.6	7.9	17.9	11.3	10.1	10.6	52.2	52.7	52.9
21	TWTCH 05	58	191.0	213.5	38.1	8.4	252.0	158.5	114.5	51.5	10.0	44.8	9.2	37.3	10.8	11.2	9.1	52.6	54.1	50.1
22	TWTCH 06	73	178.0	229.0	39.7	10.0	235.0	144.5	121.0	55.0	6.8	47.4	8.4	25.4	10.5	9.2	11.0	51.9	52.0	54.1
23	TWTCH 07	61	185.0	242.0	42.2	9.3	253.0	175.0	133.5	66.0	10.0	54.1	9.6	43.3	10.6	12.2	10.8	51.4	53.8	53.8
Mean		64	186.4	227.7	41.2	9.1	249.1	159.1	128.6	57.5	8.4	49.4	8.8		10.8	10.7	10.4	51.8	53.5	53.0
24	PAC 981	71	116.6	239.9	36.6	8.2	207.0	162.0	127.0	56.5	5.5	46.5	6.7		9.6	10.5	9.4	49.6	52.7	49.1

Note: PH−Plant height, TGFY−Total green forage yield, TDFY−Total dry forage yield, CP−Crude protein and IVOMD−In vitro organic matter digestibility. ‡ ICMV−ICRISAT Millet variety, ǂ TCHs−top-cross hybrids and † TWCHs−three-way top-cross hybrids.

Table 5. (**a**) Four best OPVs, top-cross and three-way top-cross hybrids compared for total dry forage yield (TDFY) and forage quality traits, evaluated during rainy seasons of 2018 and 2019 at ICRISAT, Patancheru; (**b**) Seven best selected cultivars of OPVs and three-way top-cross hybrids compared for total dry forage yield (TDFY) and forage quality traits, evaluated during rainy seasons of 2018 and 2019 at ICRISAT, Patancheru

S.No.	Cultivars	Total Dry Forage Yield (t ha⁻¹)		Crude Protein (CP, %)			In Vitro Organic Matter Digestibility (IVOMD, %)		
		Across Two Years (Rainy Seasons of 2018 and 2019)	% Over PAC 981 for TDFY	Across Two Years (Rainy Seasons of 2018 and 2019)		Rainy 2019	Across Two Years (Rainy Seasons of 2018 and 2019)		Rainy 2019
				First Cut	Second Cut	Third Cut	First Cut	Second Cut	Third Cut
(**a**)									
Open-pollinated varieties									
1	ICMV † 1708	9.2	37.6	11.4	9.1	9.1	51.7	51.9	50.1
2	ICMV 1617	8.3	23.9	10.1	10.4	12.5	51.6	54.2	54.9
3	ICMV 1701	8.3	23.9	11.4	10.8	11.2	51.0	53.0	50.6
4	ICMV 05555	8.1	20.9	10.0	10.3	9.5	51.7	52.6	51.4
	Mean	8.5		10.7	10.2	10.6	51.5	53.0	51.7
Top-cross hybrids									
5	TCH ǂ 01	8.9	32.8	9.6	9.7	8.9	50.7	51.1	50.3
6	TCH 02	8.9	32.8	9.1	10.0	12.6	51.2	52.0	55.6
7	TCH 03	7.9	17.9	10.4	9.7	8.3	50.9	52.2	49.5
8	TCH 04	7.6	13.4	8.1	9.5	10.3	49.1	52.3	52.2
	Mean	8.3		9.3	9.7	10.0	50.5	51.9	51.9
Three–way top-cross hybrids									
9	TWTCH ‡ 07	9.6	43.3	10.6	12.2	10.8	51.4	53.8	53.8
10	TWTCH 03	9.5	41.8	11.3	10.2	9.4	50.8	54.3	53.3
11	TWTCH 05	9.2	37.3	10.8	11.2	9.1	52.6	54.1	50.1
12	TWTCH 02	8.8	31.3	10.7	10.2	10.6	50.7	53.6	53.6
	Mean	9.3		10.9	11.0	10.0	51.4	54.0	52.7
	Check (PAC 981)	6.7		9.6	10.5	9.4	49.6	52.7	49.1
(**b**)									
Open-pollinated varieties									
1	† ICMV 1708	9.2	37.3	11.4	9.1	9.1	51.7	51.9	50.1
2	ICMV 1617	8.3	23.9	10.1	10.4	12.5	51.6	54.2	54.9
3	ICMV 1701	8.3	23.9	11.4	10.8	11.2	51.0	53.0	50.6

4	ICMV 05555	8.1	20.9	10.0	10.3	9.5	51.7	52.6	51.4
5	ICMV 1707	7.9	17.9	11.7	9.7	8.5	52.1	54.0	49.8
6	ICMV 1602	7.7	14.9	10.4	11.1	9.4	51.6	52.2	50.9
7	ICMV 1605	7.7	14.9	10.2	11.4	9.9	51.3	53.8	49.9
	Mean	8.2		10.7	10.4	10.0	51.6	53.1	51.1
Three−way top-cross hybrids									
8	‡ TWTCH 07	9.6	43.3	10.6	12.2	10.8	51.4	53.8	53.8
9	TWTCH 03	9.5	41.8	11.3	10.2	9.4	50.8	54.3	53.3
10	TWTCH 05	9.2	37.3	10.8	11.2	9.1	52.6	54.1	50.1
11	TWTCH 02	8.8	31.3	10.7	10.2	10.6	50.7	53.6	53.6
12	TWTCH 06	8.4	25.4	10.5	9.2	11.0	51.9	52.0	54.1
13	TWTCH 04	7.9	17.9	11.3	10.1	10.6	52.2	52.7	52.9
14	TWTCH 01	7.8	16.4	10.2	11.7	11.4	52.7	53.6	53.1
	Mean	8.8		10.8	10.7	10.4	51.8	53.5	53.0
	Check (PAC 981)	6.7		9.6	10.5	9.4	49.6	52.7	49.1

Note: † ICMV−ICRISAT millet variety, ‡ TCH−top-cross hybrid, and ‡ TWTCH−three-way top-cross hybrid.

4. Conclusions

The present study indicated the existence of large variability among pearl millet OPVs, top-cross and three-way top-cross hybrids for forage yield and quality traits. Higher and/comparable forage yield with better forage quality in three-way top-cross hybrids, better opportunities to broaden the genetic base of hybrids, higher adaptive potential to diverse agro−climatic conditions and lower hybrid seed production cost as compared to single-cross and top-cross hybrids suggested three-way top-cross hybrids to be the better pearl millet cultivar option for forage in arid and semi-arid conditions to feed livestock.

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