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Table of Contents: Volume 9 Number 38 18 September, 2014

ARTICLES

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Full Length Research Paper

Yield, maturation, and beverage quality of arabica coffee progenies under selection in Rondonia state, Brazil

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In this paper, we studied coffee bean yield, maturation, and other characteristics of special progenies of Arabica coffee (*Coffea arabica* L.) grown under high temperatures in a low altitude region in the state of Rondonia, Brazil. We evaluated 29 progenies developed by the Instituto Agronômico de Campinas – IAC, namely, 24 F_2BC_2 progenies of Obatã (*C. arabica* with introgressions of *C. canephora*) x (*C. eugenioides* 4n x *C. arabica*), three F_3 progenies of Catuaí x Glaucia, and two H419 lines. Seven cultivars were used as controls. A randomized block design was used with three replications, spacing of 3.0 × 1.0 m, and ten plants per plot. All the crop seasons showed a significant difference for the hulled coffee yield trait. In combined analysis, significant difference was detected among progenies, among controls, and in the progeny vs control contrast. In the average of the four harvests, hulled coffee yield was 29.30 bags ha^{-1}. The F_2 progeny Obatã x (Catuaí x EUG DP x MN) C.1594 stood out from the others with a mean yield of 47.37 bags ha^{-1}. The cultivars received beverage scores from 40 to 62, "rioysh" to "hard" beverage classification, while the progenies had scores from 40 to 80, "rio" to "barely soft" beverage classification. In regard to the maturation cycle, eleven progenies were late (April), eleven intermediate (March), and seven early (February). For continuity of advancement of generations, 104 plants were selected, derived from 22 progenies with the best productive performance, late maturation cycle, and good beverage quality.

Key words: *Coffea arabica* L., coffee plant breeding, heat tolerance.

INTRODUCTION

The estimate for the production of the coffee crop (Arabica and Canephora species) in 2014, indicates that Brazil will harvest 44.57 million bags of 60 pounds of processed coffee. With this result this season breaking the trend of growth of production, since the harvest of 2005 had been watching us the highs biannuality (annual

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alternation between large and small productions), including getting down the last harvest that was low (Conab, 2014). The result represents a reduction of 9.33% or 4.58 million bags compared with the production of 49.15 million bags obtained in the preceding cycle. Arabica represents 72.4% of total production coffee country. According to Conab, the reduction is due to the severe drought seen in the first months of 2014 and the reversal of biannuality in some producing regions.

Coffee consumption in the world in 2020 will be 166.10 million bags, which represents a 17% increase compared to the 2012 production of 142 million bags. Brazil is currently the largest producer and exporter of coffee beans, achieving a record harvest of 49.2 million bags in 2013. Among several factors, the high temperatures have limited the growth and expansion of Arabica coffee in various countries, including Brazil. The *C. arabica* species reveals its greatest potential at mean annual temperatures ranging from 18 to 23ºC. Above this range, innumerable physiological disorders are observed in the flowers and fruits of the coffee plant, resulting in yield losses and poor beverage quality. The incidence of high temperatures leads to early maturation of the fruits, resulting in loss of quality (Camargo, 1985; Damatta and Ramalho, 2006). High air temperatures during flowering, associated with an extended dry season, results in aborted flowers and, consequently, significant yield reductions (Fazuoli et al., 2007).

Due to the high temperatures registered in most of the state of Rondonia, near 26ºC, all the coffee grown in the state is of the *C. canephora* species (Robusta coffee), Arabica coffee not being represented in production. All the Arabica coffee consumed in Rondonia and other states of the North region is imported from typical production regions such as Minas Gerais and São Paulo (Souza and Santos, 2009). Since 98% of the production of the state is composed of Robusta coffee beans, local industries produce coffees with a minimum percentage of Arabica coffee. Many coffees available to the local consumer are practically 100% Robusta coffee. In North region, the per capita annual consumption is 4.80 kg of roasted coffee, and there is an estimated demand of 1.66 million bags. Aiming to meet this demand only with blends,would be necessary 830 thousand bags of Arabica coffee for the composition, considering a 50% Arabica / 50% Robusta percentage. In addition, there is the perspective of an increase in coffee consumption and in the specialty and gourmet coffee market (Abic, 2013).

Currently, there are no Arabica coffee cultivars recommended for the state of Rondonia, where the genetic materials used are of unknown origin, or even cultivars from other regions that were introduced without recommendation. In contrast, the perception of an increasing demand for Arabica coffee in the region, associated with growth in the specialty and gourmet coffee market, accounts for the interest in developing a cultivar adapted to the edaphic and climatic conditions of the region.

The present study reports the yield, maturation cycle, and beverage quality of progenies especially selected in the IAC for adaptation to the conditions of the state of Rondonia.

MATERIALS AND METHODS

The experiment was set up in September 2005 in the experimental field of Embrapa in the municipality of Ouro Preto do Oeste, RO, Brazil, geographic coordinates of 10º44'53"S and 62º12'57"W. The climate is classified as Tropical Rainy, Aw (Köppen classification), with a mean annual temperature of 25.8°C and mean annual rainfall of 2,000 mm. Mean altitude of the region is 240 m and relative air humidity is around 82% most of the year.

A total of 36 Arabica coffee genotypes were evaluated, consisting of 29 progenies and seven control cultivars. The progenies are derived from hybridizations carried out in the Instituto Agronômico de Campinas – IAC, Campinas, SP, Brazil (Medina Filho et al., 2012), from late maturity cultivars of high yield performance and good beverage quality. From these crosses, 126 backcrossed hybrids of Obatã (BC_2) with *C. arabica* x (*C. eugenioides* 4n x *C. arabica*) were obtained. Under study in Campinas, 24 of them were selected for very late maturity (F_2BC_2), characteristic of *C. eugenioides*, desirable for the tropical conditions of Rondonia. Three F_3 progenies of Catuaí x Glaucia and two H419 lines were also selected since they exhibited late maturity. The cultivars Bourbon Amarelo, Catuaí Amarelo, Catuaí Vermelho IAC 15, Obatã, Obatã Vermelho (late), Oeiras, and Topázio MG 1190 were used as controls.

A randomized block experimental design was used with three replications, ten plants per plot, and spacing of 3.0 × 1.0 m. The experiments were evaluated during four crop season (2007/2008, 2008/2009, 2009/2010 and 2011/2012) and conducted according to the fertilization recommendations coffee. The normal management practices used for the crop were adopted. Problems in the release of funds in 2010 made it unfeasible the cultivation and harvesting of the 2010/2011 season, making it impossible to collect data. Yield was assessed in 60 kg bags of hulled coffee per hectare (bags ha^{-1}). Harvest was carried out in individual plots, measured in liters of "café da roça" (coffee fruits of mixed maturity) per plot. Subsequently, the volume of coffee harvested was converted to bags ha^{-1}, considering the mean yield of a bag of 60 kg of hulled coffee for each 480 L of "café da roça". This yield corresponds to the regional average.

The fruit maturation of the *C. arabica* species in climatic conditions of Rondonia usually occurs in february. Thus, the maturation cycle of the plants was determined considering the interval of 20 to 30 days between harvests. The criterion for classification: Early (E) in february; Intermediate (I) in march; Late (L) in april.

The percentage of poorly filled fruits was obtained from an initial sample of one liter of coffee from which one hundred fruits in the cherry stage were deposited in a container with water to count the number of floater fruits (Medina Filho and Bordignon, 2003).

For the evaluations of beverage quality, samples were harvested manually from four different points of the middle upper third of the plants. The collection period was from the months of February to April, before the beginning of harvest, depending on the degree of maturity of the plot. A manual pulper with continual water flow was used for processing. The coffee was dried on a covered cement yard, until reaching a moisture level of approximately 11%. The dried samples were hulled and placed in paper packaging until analysis. Sensory analysis of the beverage quality was performed by two cuppers, with only one determination or cupping per cupper

Table 1. Summary of combined analysis of variance and estimates of heritability (h^2) and selective accuracy ($\hat{r}_{\hat{g}g}$) for hulled coffee yield (bags ha^{-1}) in regard to the 2007/2008, 2008/2009, 2009/2010, and 2011/2012 crop seasons (Ouro Preto do Oeste, Rondonia, Brazil, 2013).

SV	DF	Mean square
		Hulled coffee(bags ha^{-1})
Blocks	2	408.9916
Treatments	35	567.3459**
Progenies	28	575.5037**
Controls	6	419.1006**
Progenies vs. controls	1	1228.4018**
Crop seasons	3	37331.4234**
Treatments x crop seasons	105	192.2146**
Progenies x crop seasons	84	200.3860**
Controls x crop seasons	18	146.9110**
Progenies vs. Contr. x crop seasons	3	235.2379*
Residue	286	69.6340
Overall mean	29.30	
Heritability (h^2)	87.73	
Selective Accuracy ($\hat{r}_{\hat{g}g}$)	81.32	

**, *: Significant, at 1 and 5% by the F test, respectively.

for each sample. Each sample composed of three cups was analyzed for sensory characteristics by the protocol of the Specialty Coffee Association of America (SCAA, 2014) in regard to aroma, clean cup, uniformity, sweetness, body, acidity, flavor, aftertaste, balance, and overall impression. The combination of these properties determined the value of the overall score of each sample.

The yield data of hulled coffee were subjected to analysis of variance with the significance of the effects verified by the F test at 5% probability. Selective accuracy ($\hat{r}_{\hat{g}g}$), determined by means of the expression $\hat{r}_{\hat{g}g} = (\mathrm{F}-1/\mathrm{F})^{1/2}$ (in which F is the value of the F test of Snedecor for the genotype effect (Resende and Duarte, 2007), was estimated to check experimental precision. Analyses of variance and gain in selection were carried out using the computational software Genes (Cruz, 2013).

RESULTS

Combined analysis of all the crop seasons shows a significant difference among treatments for the hulled coffee yield characteristic (Table 1). The same result was observed among progenies and among controls, manifesting the large genetic variability among the genotypes evaluated. The analyses among crop seasons also exhibited significant differences for yield.

The progeny vs. control contrast was also significant for hulled coffee yield, indicating that the progenies and controls had different behaviors for this characteristic (Table 1). The breakdown of the sum of squares of the progeny x crop season source of variation indicated significance for yield, showing that the behavior of the progenies was not the same in all the harvest seasons (Table 1). The significant progeny vs. control x crop season interaction for hulled coffee yield indicated that the performance of the progenies was different from that of the controls, in all the crop seasons. In Table 2, the mean values of the progenies and of the controls are indicated, where it may be observed that most of the progenies were greater than the controls in coffee bean production. All the crop seasons showed selective accuracy estimates of high magnitude (75.25% < $\hat{r}_{\hat{g}g}$< 91.79%), indicating good experimental precision. The lowest mean yield was observed in the 2007/08 crop season (8.61 bags ha^{-1}).

The low yield is due to the fact that this was the first production. In the 2008/09 crop season, the overall mean value was 18.93 bags ha^{-1}. In 2009/2010 and 2011/2012, satisfactory yields were obtained, with mean values of 48.05 and 41.55 bags ha^{-1}, respectively. These yields obtained in the experimental field studied are expressive if we consider the geographic location, latitude, and local climatic conditions of low altitude and high temperatures (Figure 1). The mean value of the four harvests was 29.30 bags ha^{-1} (Table 2), and the mean value of the progenies was 30.11 bags ha^{-1}, 14% above the mean value of the controls (25.85 bags ha^{-1}). It should be noted that 22% of the genotypes exhibited yields above 35 bags ha^{-1}, among which the F_2 progeny Obatã x (Catuaí x EUG DP x MN) C.1594 stands out, with a mean value of 47.37 bags ha^{-1} (Table 2). It is interesting to note that the heritability estimated for hulled coffee yield of this experimental field was 87.73% (Table 1), stimulating subsequent selections. The overall

Table 2. Mean estimate of the agronomic traits of 29 progenies and seven cultivars of *Coffea arabica* in regard to hulled coffee yield (bags ha^{-1}), percentage of poorly filled fruits (%), maturation cycle, and color of the fruits for the crop seasons of 2007/2008, 2008/2009, 2009/2010, and 2011/2012. Ouro Preto do Oeste, Rondonia, Brazil, 2013.

Progenies	Hulled coffee (bags ha^{-1})	Poorly filled fruits (%)	Maturation cycle[1]	Fruit color[2]
Progenies				
F_2 Obatã x (Catuaí x EUG DP x MN) C.1594	47.37	13	L	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1595	38.73	19	I	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1597	38.38	12	E	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1575	37.68	18	E	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1526	36.98	21	I	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1576	36.40	10	L	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1627	35.93	16	I	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1578	35.23	18	L	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1528	33.02	14	I	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1560	32.32	20	I	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1608	32.20	16	L	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1518	31.73	22	L	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1583	31.50	21	I	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1585	31.38	10	I	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1552	31.03	18	L	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1633	30.92	19	L	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1515	30.45	20	I	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1564	29.87	9	I	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1587	27.88	23	L	R
F_3 Catuaí x Gláucia C.277	27.30	17	E	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1551	26.13	23	I	R
H419-10-6-2-10 (Paraíso - Sítio Jatobá)	25.32	16	E	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1562	22.28	26	E	R
F_3 Catuaí x Gláucia C.175	21.47	17	E	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1605	21.23	14	L	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1599	20.07	10	I	R
H419-10-6-2-3-27 (Canteiro 5 - 23)	20.07	10	E	Y
F_3 Catuaí x Gláucia C.182	19.72	12	L	R
F_2 Obatã x (Catuaí x EUG DP x MN) C.1635	19.48	19	L	R
Cultivars				
Obatã Vermelho Tardio (Prop. Altair)	35.93	10	E	R
Obatã	30.33	22	I	R
Bourbon Amarelo	26.95	10	E	Y
Catuaí Vermelho IAC 15	23.92	16	E	R
Catuaí Amarelo	21.47	15	E	Y
Topázio MG 1190 (Canteiro 8 – 23)	21.35	12	E	R
Oeiras (Canteiro 5 – 33)	19.95	22	E	R
Overall Mean	29.30	16.4		
Mean of progenies	30.11			
Mean of controls	25.85			

[1]E = Early; I = Intermediate; L = Late. [2]R = Red; Y = Yellow.

percentage of poorly filled fruits was 16.4%; the mean of the cultivars was 15.3%, and of the progenies 16.7% (Table 2). In the years evaluated, there was no incidence of coffee rust, probably due to the high temperatures

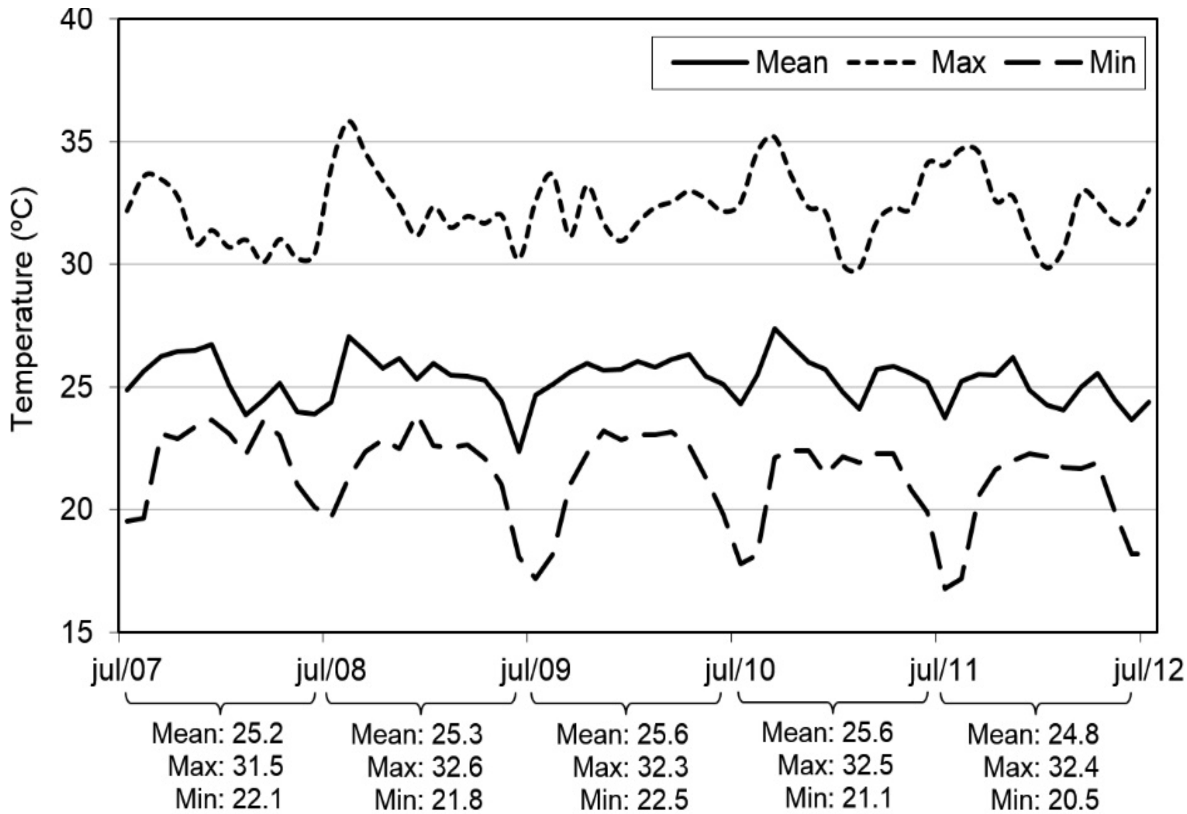

Figure 1. Monthly representation of mean, maximum, and minimum temperatures during the period from July/2007 to July/2012 in Ouro Preto do Oeste, Rondonia. Mean, maximum, and minimum temperatures are also represented by crop seasons (2007/2008, 2008/2009, 2009/2010 and 2011/2012) (Ouro Preto do Oeste, Rondonia, Brazil, 2013).

throughout the crop cycle.

Evaluation of four crop seasons of production allowed the reliability of the genetic parameters in each set of crop seasons to be verified (Table 4). It is fitting to note that only the evaluation of the 1st and 2nd crop season would already be sufficient to estimate the genetic parameters with efficiency, with a coefficient of determination (R^2) of 74.68%. With the inclusion of the 3rd harvest, the value of the R^2 estimates changed little, reaching 78.37%. This information is important because in breeding programs like the one carried out, in which there is the need for advancing generations, the earlier selection is performed, the more dynamic the process of obtaining lines is. In relation to the maturation cycle, it was observed that all the harvests were carried out from the months of February to April. In regard to the difference between progenies, eleven were classified as late cycle (April), 11 as intermediate cycle (March), and 7 as early cycle (February) (Table 2). The control cultivars exhibited an early maturation cycle, except for Obatã, with an intermediate cycle.

Sensory analyses showed that the scores for Overall Quality and SCAA values of the cultivars ranged from 1 to 3 and from 40 to 58, respectively, with beverage types from rioysh to hard (Table 3). The estimates of the same parameters for the progenies proved to be more interesting, with values ranging from 1 to 3.5 and 40 to 80, with beverage types from hard to barely soft.

DISCUSSION

The high accuracy observed in analysis of all the crop seasons shows that the experimental field was well conducted in the various crop years, providing good reliability to the genotypic values obtained for hulled coffee yield. The use of accuracy as a measure of experimental precision, suggested by Resende and Duarte (2007), has the advantage of not depending on the mean, which provides greater security in the use of phenotypic expression as an indicator of genotypic variation. Accuracy values greater than 70% indicate high experimental precision.

The productive performance of the progenies evaluated under the climatic conditions of Rondonia are very similar to the observations in trials for evaluation of progenies conducted by Carvalho et al. (2010) and Botelho et al. (2007) in the southern region of Minas Gerais. Thus, the

Table 3. Estimate of the sensory analyses of 29 progenies and seven cultivars of *Coffea arabica* in regard to Density, Type of beverage, Residual Flavor, Q Global and SCAA, in reference to the 2009/2010 crop year (Ouro Preto do Oeste, Rondonia, Brazil, 2013).

Progenies	Density	Type of beverage	Residual flavor	Q Global	SCAA
Progenies					
F_2 Obatã x (Catuaí x EUG DP x MN) C.1594	1.02	Hard	Harsh/sweet	3	65
F_2 Obatã x (Catuaí x EUG DP x MN) C.1595	1.03	Barely soft	Desir/unbalanced	3	64
F_2 Obatã x (Catuaí x EUG DP x MN) C.1597	1.02	Hard	Astringent	2	56
F_2 Obatã x (Catuaí x EUG DP x MN) C.1575	1.03	Hard	Dirty	1.5	56
F_2 Obatã x (Catuaí x EUG DP x MN) C.1526	1.01	Hard	Harsh	3	60
F_2 Obatã x (Catuaí x EUG DP x MN) C.1576	1.03	Hard	Harsh/acid	2	60
F_2 Obatã x (Catuaí x EUG DP x MN) C.1627	1.03	Barely soft	Des/harsh/sweet	3.5	66.5
F_2 Obatã x (Catuaí x EUG DP x MN) C.1578	1.01	Hard	Und/harsh/bitter	2	60
F_2 Obatã x (Catuaí x EUG DP x MN) C.1528	1.00	Barely soft	Desirable/sweet	3.5	65
F_2 Obatã x (Catuaí x EUG DP x MN) C.1560	1.02	Hard	Harsh	3	67
F_2 Obatã x (Catuaí x EUG DP x MN) C.1608	1.02	Barely soft	Des/sweet/astr	3.5	66
F_2 Obatã x (Catuaí x EUG DP x MN) C.1518	1.02	Hard	Harsh	2	51
F_2 Obatã x (Catuaí x EUG DP x MN) C.1583	1.01	Barely soft	Des/sweet/wodef	3	65
F_2 Obatã x (Catuaí x EUG DP x MN) C.1585	1.00	Hard	Harsh/bitter	2.5	56
F_2 Obatã x (Catuaí x EUG DP x MN) C.1552	1.04	Barely soft	Citri/bodied/sweet	4	80
F_2 Obatã x (Catuaí x EUG DP x MN) C.1633	1.01	Hard	Harsh/astr	2.5	60
F_2 Obatã x (Catuaí x EUG DP x MN) C.1515	1.00	Hard	Undesir/strange	1	51
F_2 Obatã x (Catuaí x EUG DP x MN) C.1564	1.02	Hard	Undesir/astr	1.5	57
F_2 Obatã x (Catuaí x EUG DP x MN) C.1587	1.01	Hard	Harsh	2	57
F_3 Catuaí x Gláucia C.277	0.99	Hard	Undesir	2	52
F_2 Obatã x (Catuaí x EUG DP x MN) C.1551	1.03	Barely soft	Des/bodied/sweet	4	80
Paraíso (H419-10-6-2-10 - Sítio Jatobá)	1.03	Hard	Harsh	3	60.5
F_2 Obatã x (Catuaí x EUG DP x MN) C.1562	1.01	Hard	Astr/acid	2.5	62
F_3 Catuaí x Gláucia C.175	1.01	Hard	Dirty	2	56
F_2 Obatã x (Catuaí x EUG DP x MN) C.1605	1.03	Rioysh	Und/dirty	1	40
F_2 Obatã x (Catuaí x EUG DP x MN) C.1599	1.02	Hard	Harsh	2	58
H419-10-6-2-3-27 (Canteiro 5 – 23)	1.02	Hard	Undesirable	1.5	53
F_3 Catuaí x Gláucia C.182	1.01	Hard	Harsh	2	55
F_2 Obatã x (Catuaí x EUG DP x MN) C.1635	1.02	Barely soft	Des/bodied	3.5	65
Cultivars					
Obatã Vermelho Tardio (Prop. Altair)	1.03	hard	Harsh/unbal	3	58
Obatã	1.03	hard	Harsh/green	2	56
Bourbon Amarelo	1.05	rioysh/rio	Undes/chemical	1	40
Catuaí Vermelho IAC 15	1.01	hard	Desirable	3	62
Catuaí Amarelo	1.00	hard	Undesir	2	55
Topázio MG 1190 (Canteiro 8 – 23)	0.98	hard	Harsh	2	57
Oeiras (Canteiro 5 – 33)	0.98	hard	harsh	2	53.5

results obtained showed that growing of Arabica coffee in the region of Rondonia is promising and provides technical and scientific information that stimulate the progress of the breeding program with the selection of new progenies. The estimate of heritability near 87% corroborates this statement since it indicates the proportion of genetic variance relative to total phenotypic variance, that is, the inheritable proportion of the total variability (Mohsin et al., 2009).

On the maternal side, the gene pool of these progenies is derived from the cultivar Obatã, which is highly productive, but demanding in nutrition and water. It has quite a late cycle in the conditions of the Southeast of Brazil and has resistance to various strains of coffee rust, introgressed characteristics of *Coffea canephora.* On the paternal side, part of the gene pool is derived from the species *Coffea eugenioides* which, under the conditions of Campinas, is also quite late and has good beverage

Table 4. Estimate of the coefficient of determination (R^2) in relation to the number and time period of harvests.

No. of harvests	Crop seasons	Component	R^2
2	1st and 2nd	0.5959	74.68
2	2nd and 3rd	0.4774	64.63
2	3rd and 4th	0.4475	61.83
3	1st, 2nd, and 3rd	0.5470	78.37
3	2nd, 3rd, and 4th	0.3270	59.31
4	All the crop seasons	0.3980	72.56

Values in reference to sets of two, three, and four crop seasons (Ouro Preto do Oeste, Rondonia, Brazil, 2013).

quality, characteristics which segregate in its progenies of backcrosses for *C. arabica* and which were especially selected so as to take advantage of the edaphic and climatic conditions of Rondonia. This study reflects the importance not only of preservation of the genetic resources of *Coffea* and the like, but also the effective use of genetic variability in coffee breeding, meeting the agroindustrial demands of the crop and the regional socioeconomic needs.

The same control cultivars studied here were evaluated by Teixeira et al. (2013) in a competitive trial of cultivars in the municipality of Ouro Preto do Oeste, RO, during the same period. It was observed that the yields and maturation cycles were very similar, indirectly indicating good reliability of the data obtained, which confirm the low adaptation of traditional Arabica cultivars and the superiority of diverse progenies studied in regard to production, late maturation, and beverage quality. Several of them proved to be productive, late, and with good beverage quality, some obtaining 80 points and Overall Quality 4, indices not seen before for Arabica coffees in Rondonia.

Apparent density did not show expressive relationships with other properties, likewise for roasting and its uniformity. Without great differences or restrictions, most of the genotypes exhibited reasonable body, acidity, and bitterness and, with rare exceptions, hard beverage to better, with quite variable residual flavors, some indicative of incomplete maturation and others with desirable characteristics that affected the values of Overall Quality and of the SCAA score. Acidity, which was generally agreeable, exhibited citric profiles of orange or lime. Chocolate notes were present in almost all the samples. The beverage of some progenies also stood out through marked mildness, absent in the beverage of all the cultivars.

In no progeny was the percentage of floaters excessive, a result similar to that found by Botelho et al. (2010) in trials in the southeast of Brazil, indicating that this characteristic was not affected by the high temperatures of Rondonia. The yield of the progenies was the main criterion used to measure their tolerance to thermal stress. Wahid et al. (2007) affirm that heat tolerance is the capacity of the plant to develop and produce under high temperature conditions. Falconer and Mackay (1996) show that the grain yield trait is governed by various genes of small effect on the phenotype, indicating that this trait is quantitative, strongly affected by the environment.

According to Bardin-Camparotto et al. (2012), the thermal factor has an effect on the maturation cycle of the coffee fruits, which when subjected to thermal stress conditions tend to exhibit an earlier maturation cycle. Late maturation was also an important selection criterion. High incidence of rains in the state of Rondonia occurs during the months of October to March. Thus, cultivars with maturation after this period facilitate harvest, drying in the yard, and promotion of better beverage quality.

To proceed with the breeding plan with a view toward the development of varieties of *C. arabica* adapted to Rondonia and similar regions, individual selection of plants was carried out taking into consideration the genealogical distribution, the characteristics of the progenies, and the individual properties of the plants in relation to the others of the progeny. Thus, 104 plants were selected, deriving the F_3BC_2 generation. The plants, genealogically, are derived from 22 progenies elected for their productive performance, late maturation cycle, and good beverage quality. Figure 2 schematically indicates the annual productive performance of the five best progenies selected.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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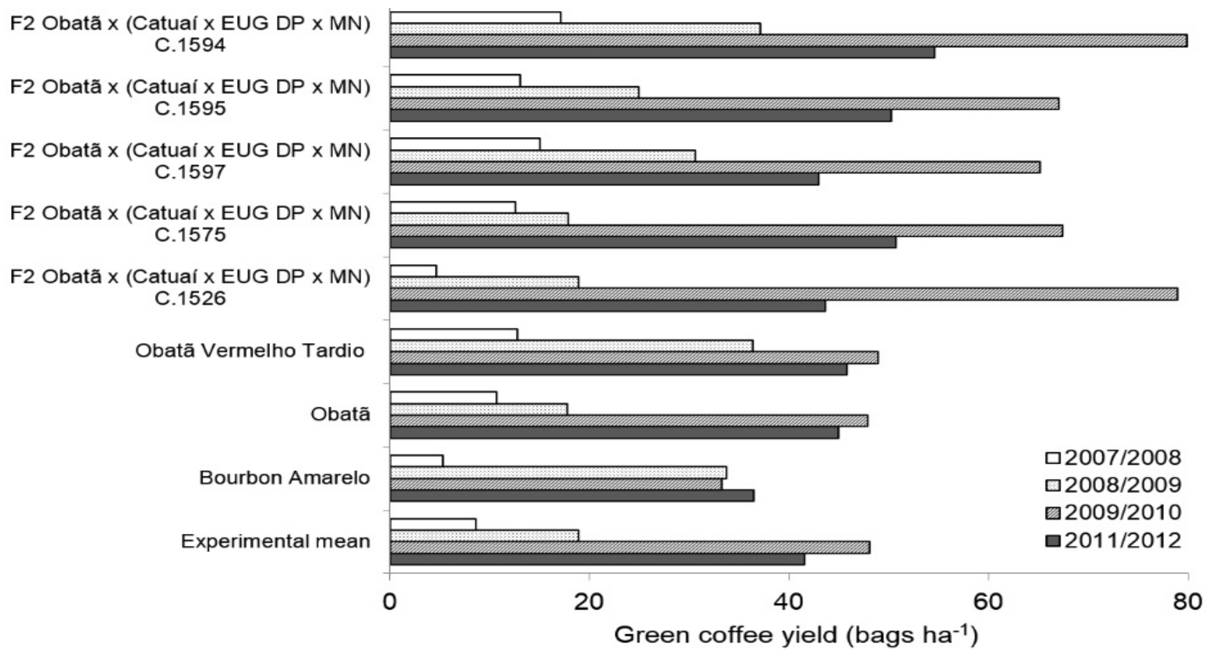

Figure 2. Annual performance of five Arabica coffee progenies and three cultivars that showed the best yield during the 2007/2008, 2008/2009, 2009/2010, and 2011/2012 crop seasons (bags ha^{-1}) (Ouro Preto do Oeste, Rondonia, Brazil, 2013).

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Significance of vinasses waste management in agriculture and environmental quality- Review

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Vinasse is a waste material from distillery industries which has lot of organic and inorganic loads. It is utilized in agriculture for cheap nutrients source, ameliorating agents and animal feed beyond the limitation of high biochemical oxygen demand (BOD; 46100 to 96000 mgL^{-1}), chemical oxygen demand (COD; 104000 to 134400 mgL^{-1}) and total dissolved salts (30.5 to 45.2 dSm^{-1}) content even after the pollutant removal treatments. Vinasse treatments with combined approach of aerobic and anaerobic methods are more effective by both cost and pollutant on removal efficiency. Optimized dose of vinasse application has significance over soil properties, crop qualities and yield improvement. Globally, it has high potential to substitute potassium and nitrogen nutrients to the present level of annual consumption. It also contributes a substantial amount of phosphorous, calcium, sulphur and micronutrients to crops. In developed countries, starchy vinasse used as animal feed on a lean season to animals that improved the feed digestibility and animal quality where feed shortages are experienced. However, inadequate knowledge of vinasse properties and mode of utilization in agriculture questioned environmental quality for ground water pollution. Further research is needed to be strengthened for increased pollutant removal efficiency and diversified utilization in different cropping system (temporal scale) for effective utilization and safe disposal to the ecosystem. This article aimed to give the vinasse kinds and their characteristics features, soil-vinases interaction mechanism and its influence on plant, soil and water quality as consequence in agriculture.

Key words: Chemical oxygen demand (COD), biochemical oxygen demand (BOD), eco-friendly, utilization, environmental quality.

INTRODUCTION

Vinasse is a waste material from distillery industries which has potential to cause major environmental problems across the world. It is also referred to as stillage or molasses spent wash in Asian countries (Sajbrt et al., 2010; Muhammad et al., 2012). More than 95% of ethanol production comes from sugar and starch materials based industries and it is contributing 42 and 58% to the total production, respectively (Tolmasquim, 2007). An average of 12 to 15 ft^3 vinasse per ft^3 of ethanol is produced and the absolute production anticipated increase with changing market scenario and government policies on ethanol production and fuel

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Table 1. Continent wise annual vinasse potential share of plant primary nutrients sources to its consumption in 2010.

S/N	Continents	Vinasse production (billion litres)	Nutrient content in vinasse (g/L)			Potential Share of N, P_2O_5 and K_2O to nutrient consumption* (%)			References
			N	P_20_5	K_2O	N	P_2O_5	K_2O	
1	North and Central America	844.0	1.34	1.41	7.26	7.00	25.07	119.84	Gamboa et al. (2011)
2	South America	644.2	0.35	0.24	1.54	3.50	2.66	17.81	Alexander et al. (2006)
3	Europe	66.3	32.50	0.26	34.0	15.8	0.49	54.34	Tejada and Gonzalez (2006)
4	Asia	82.01	5.30	0.20	11.4	0.60	0.07	6.27	Jiang et al. (2012)
5	Australia	4.9	1.83	ng	1.01	0.70	ng	2.64	Carmen (2006)
6	Africa	2.1	0.83	1.11	0.22	0.10	0.11	0.12	Musee et al. (2007)

*International Fertilizer Industries Association (2010) and Food and Agriculture organization (2010), **ng,** Negligible.

utilization (Renewable Fuels Association, 2011). In 2008, the global ethanol production is 79 billion litres and it approximately generates vinasse of 2.4 × 10^{12} L (Carmen, 2006; Krzywonos et al., 2008). According to Renewables Fuel Association Report (2011), approximately 67% of the global ethanol production used for fuel and the same data used here confirm the status of vinasse production and mineral nutrient supply potential to agriculture (Table 1). The vinasse had been disposed directly to the flowing water that affected water quality, ecosystem and human health but only few countries improved upon these issues by making stringent amendments and still many counties have a long way to go about it (EPA, 2012). An alternate management to control the water pollution, significant proportion of vinasse have been disposed to agriculture for their manure value even though it has potential for other commercial utilization like production of single cell protein, glycerol, enzymes, astaxanthia, hormones, bio polymers and organic acid (Nataraj et al., 2006).

Here, the term environmental quality is taken as change in properties and function of environmental components of soil, plant and animal as consequence of vinasses application in agriculture (Johnson et al., 1997). Large proportion of lands are degraded either directly or indirectly by faulty practice of cultivation, grazing etc. that limit the soil and plant capacity and eventually food security of the world (IILRI, 1988). Vinasse have been utilized in large area of agriculture by time and space, now it is need to assess their significance and/or impacts an environmental components for eco-friendly utilization as well as safe disposal to agriculture and effectively manage the challenges in handling of vinasses in the upcoming years.

VINASSE CHARACTERIZATION

Vinasse is a by-product of distillery industries produced mostly from raw materials of corn, wheat, rice, potatoes, sugar beets, sugarcane and sweet sorghum. Its composition (Table 2) varied considerably from one distillery to another for raw materials and stages at which it is used in the process, fermentation type and adopted distillation process (Bustamante et al., 2005; Krzywonos et al., 2009). It is light-brown liquid which contains basically of water (93%) and organic solids and minerals (7%) that condensed to 60% (on wet basis) dry matter (Gemtos et al., 1999). Sugarcane vinasse is produced from juice and often with molasses, has more concentration of carbon, potassium, phosphate, sulphate, calcium, iron, sodium and other micronutrient than former (Gopal and Kammen, 2009) and has lot of organic compounds such as glycerol, lactic acid and acetic acid (Decloux et al., 2002).

Similarly, sugar beet vinasse has significant amount of organic compounds like glycerol, betaine, oxalate, lactate and malate (Parnaudeau et al., 2006). Grapes vinasse has low nitrogen in comparison to sugarcane and sugar beet vinasse, however, rich in potassium, organic acids such as tartaric acid, malic acid, succinic acid, acetic acid and lactic acids, sugars, soluble proteins and phenolic compounds (Vlyssides et al., 2005; Melamane et al., 2007). Vinasse of corn and sweet sorghum are rich in nitrogen, phosphorous, potassium, sodium and amino acids of alanine and proline (Resende et al., 2005; Rausch and Belyea, 2006).

Usually, vinasses are characterized by low pH (4.5 to 5.5), high salts (30.5 to 45.2 dSm^{-1}), high biochemical oxygen demand (BOD; 46100 to 96000 mgL^{-1}) and chemical oxygen demand (COD; 104000 to 134400 mgL^{-1}) medium to high amount of micronutrients and hormones (Mahimairaja and Bolan, 2004). Awful color in vinasse/distillery spent wash is due to

Table 2. Vinasse kinds and their chemical composition.

Parameter	Sweet sorghum	Sugarcane spent wash	Sugar beet	Wine	Wine lees	Carrot
pH	4.5	4.1	4.9	4.2	3.8	4.4
EC (dS/m)	na	22 dS/m	na	na	na	na
BOD (mg/L)	46000	50000	na	16300	67500	22750
COD (mg/L)	79900	96000	na	27500	122000	44000
OC (mg/L)	na	156000	400	21000	84000	na
N (%)	0.08	0.13	3.25	0.07	1.74	0.15
P (%)	0.2	0.00	0.03	0.01	0.74	na
K (%)	na	0.79	12.60	0.12	0.02	0.22
Ca (ppm)	na	na	39.0	12	240	na
Na (ppm)	na	na	21000	120	80	358.6
Fe (ppm)	317	12.7	226	18	25	na
Mn (ppm)	na	0.42	3.4	na	na	na
Zn (ppm)	na	0.7	12	12	21	na
Cu (ppm)	37	0.27	2.1	0.05	1.2	na
References	Wilkie et al. (2000)	Kumari and Phogat (2010)	Tejada et al. (2007)	Vlyssides et al. (2005)	Vlyssides et al. (2005)	Aimaretti et al. 2013
Country	USA	India	Spain	Greece	Greece	Argentina

recalcitrant compounds such as caramels, melanoidines and its sister products like hydroxyl methyl furfural and colloidal nature of caramels are resistant to decomposition and possess toxicity to microflora (Bustamante et al., 2005). Further, presence of putrescible organic compounds namely indol, 3- methyl indol and sulphur-containing substance causes bad odour and serious aesthetic problems (Pant and Adholeya, 2007a). Therefore, alteration of spent wash character is needed for these bad qualities before disposal to environment for the consideration of soil, plant, animal quality (Rath et al., 2010).

VINASSE TREATMENTS: AEROBIC AND ANAEROBIC METHODS

The pollutant in vinasse are attributed to various processes namely, chemical treatment, sugar production, alcohol fermentation and high quality biologically oxidisable organic matter for raw materials (Rosabal et al., 2007; Farhadian et al., 2007). High level recalcitrant organic and inorganic in vinasse are potential to pollute environment by oxygen depletion, salinity and specific ion toxicity to the environmental components (Mane et al., 2006). They have been brought down to the safe level at industry outlet by various treatments and they are categorized into aerobic and anaerobic methods (Prasad and Srivastava, 2009; Murthy and Chaudhari, 2009). Each has its own efficiency in pollutant removal and combination of practices hold good than single practice (Satyawali and Balakrishnan, 2008). According to Wolmarans and Villiers (2002) and Orendain (2006), both efficiency and cost effective treatments was opted to process vinasse starting by anaerobic treatments for high BOD, COD and total solid content, followed by some aerobic methods (Orendain et al., 2006). Bio-gas produced in the anaerobic methods could certain to substitute some extend fuel needs of industrial operation and same time it avoids the high load sludge problems to the subsequent aerobic method (Kumar and Chandra, 2006). Even after the anaerobic treatment, some organic compounds in the vinasses causes dark colour and it is removed to some extent by aerobic digestion with biological and/or physico-chemical treatments namely lagooning, coagulation, filtration, adsorption, ozonation, ion exchange, reverse osmosis, electro-flocculation, chemical oxidation, precipitation and incineration (Melamane et al., 2007). Even though biological treatments are environmental friendly and cost effective, there are some chemicals which are

Table 3. Vinasse treatment methods and their pollutants removal efficiency.

Countries	Treatments	Process	Kind of vinasses	BOD removal (%)	COD removal (%)	Color removal (%)	References
South Africa		Upflow Anaerobic Sludge Blanket (UASB) reactor	Distillery waste water	na	90	na	Wolmarans and Villiers (2002)
Germany		Continuous Stirred Tank Reactor (CSTR).	Tequila vinasse	na	90-95	na	Mendez-Acosta,(2010)
China	Anaerobic treatments	Anaerobic Sequencing Batch (ASB) Reactor	Cassava stillage	na	90	na	Luo et al. (2009)
Jappan		Kubota Submerged Anaerobic Membrane Bioreactor (KSAMBR)	Distillery waste water from barely and potato	na	92	na	Kanai et al. (2010)
Colombia		USBF Reactor	winery effluent	na	96	na	Molina et al. (2007)
India		electro-chemical methods	Vinasse	na	56	na	Krishna et al. (2010)
		Ozanation	aerobically treated vinass	na	21.5		Benitez et al. (2000)
India		Pseudomonas sp.	Vinasse-sugarcane molasses	na	63	56	Chavan et al. (2006)
India	Aerobic treatments	Pseudomonas putida and Aeromoas sp	Vinasse-sugarcane molasses	na	44	60	Ghosh et al. (2002)
UK		Phanerochaete chrysosporium	Vinasse-sugarcane molasses	85.4		85	Coulibaly et al. (2003) and Fahy et al. (1997)
India		Double coagulation by ferrous sulfate		na	46.4	82.5	Chauhan and Kumar (2012)
India		Calcium Hydroxide		na	32.1	46.4	Chauhan and Kumar (2012)
France		Anaerobic treatment-Aerobic treatment	Wine vinasse	na	99.5	na	Moletta (2005)
Brazil	Combined treatments	Anaerobic Sequencing batch reactor – Aerobic (SBR)	Domestic wate water	na	94	na	Callado and Foresti (2001)
		UASB–SBR	Domestic waste water	98	92	na	Guimaraes et al. (2003)
South Africa		USAB-Activated sludge treatment	Winery effluent	na	96.5	na	Musee et al. (2007)

toxic to microbes that need to be treated by physic-chemical methods (Fahy et al., 1997). Therefore, combination of anaerobic and aerobic methods is recommended and most commonly followed contemporary practices and their efficiency in pollutant removal given in Table 3.

UTILIZATION OF SPENT WASH IN AGRICULTURE

Beyond the limitation, it is utilized in agriculture for organic fertilizer as complement to mineral fertilizers, irrigation by mixing with good quality water, reclamation agents, neutralizing materials to compost preparation, feeding materials to animals and production of microbial protein (Linda et al., 2005; Kumari and Phogat, 2010; Rodrigo and Araujo, 2011).

With an advancement of knowledge in vinasse, handling and utilization improved an environmental quality over the past open to soil and water (Table 4) (Chhonkar et al., 2000). In India, reclamation of sodic soils by spent wash is considered for economical practice than gypsum for poor and marginal farmers (Haroon and Bose, 2004; Mahendra et al., 2010).

Therefore, vinasse rather waste, is considered as win-win situation in agriculture for safe disposal and quality improvement of soils, plants and animals (Kulkarni et al., 1987; Mahimairaja and Bolan, 2004).

SIGNIFICANCE OF VINASSE APPLICATION AND ITS MECHANISM FOR SOIL QUALITY IMPROVEMENT

Vinasse is known for high organic matter, potassium, nitrogen, calcium and micronutrients; and decades ago it was substituted for fertilizer in agriculture. In tropical countries, it is a material in agriculture for soil carbon sequestration where most of the soils are poorly fertile and has low soil organic matter content (Biswas et al., 2009). Across the world, vinasse application in agriculture have been reported positive feedback to soil properties for bulk density, porosity, water retention, stable aggregates and structure formation, infiltration, hydraulic conductivity, soil

Table 4. Vinasse practice in agriculture and major crop yield.

Countries	Crop/Cropping system	Recommended practices	Remarks	Yield increase (%)	References
Cuba	Sugar cane	Irrigation (300 m^3/ha) as 1/5th dilution of vinasses about 2 irrigation per cycle + 60:25:80 kg of N, P_2O_5 and K_2O per ha.	Main Ratoon-2	64.4 51.4	Armengol et al. (2003)
Mauritius	Sugar cane	Application of vinasse at 100 m^3/ha just prior to planting	Average yield for 4 years in 3 places	9.81	Soobadar and Kwong (2013)
India	Sugarcane	Application of PMSW* at 1 KL up to 3 months of planting + 100% N, P and K	-	63.3	Janaki and Velu (2010)
Venezuela	Sugar cane	Vinasse application at 50 m^3/ha + 80 kg N + 45 kg P_2O_5/ha	Main crop Ratoon-1 Ratoon-2	43 13 12	Gomez (2000)
India	Rice	GLM (6.5 t/ha.+ PMSW at125 m^3/ha.	-	102.8	Das et al. (2010)
Spain	Wheat	Beet vinasse fresh 6 t/ha + 150 kg of N as NH_4NO_3	-	6.1	Tejada and Gonzalez (2005)
Spain	Maize	Vinasse at 2000 l/ha+ 200:80:120 kg NP and K kg/ha	-	13.8	Tejada and Gonzalez (2006)
India	Sweet sorghum	150 kg of N through spent wash one month prior to sowing	-	61.1	Mallikarjun (2010)
India	Soybean-Wheat cropping systems	Application of molasses spent wash at 5 cm to soybean and 2.5 cm to wheat	Soybean Wheat	60.3 94.7	Biswas et al. (2009)
Egypt	Wheat	Foliar spray of vinasse at10% with 2% N, P_2O_5 and K_2O for every 10 days	-	50	Tejada and Gonzalez (2005)
India	Fodder sorghum	Rock phosphate and SW(1:20)	-	31.3	Kumari and Phogat (2012)
Spain	Corn	Grape marc 40 + 10% vinasse at 1500 kg/ha.	In comparison to farmer practices	25	Benitez et al. (2000)

*Post methanated spent wash.

reaction, nutrient availability, improved nutrient use efficiency and soil biochemical properties eventually on crop growth and developments (Pathak et al., 1999; Armengol et al., 2003). In Cuba, 40 years of irrigation practices in sugarcane crops with vinasse contained 11 to 15% of dissolved organic carbon and increased the soil organic carbon to about 40 to 500% in ultisols and similar observation have been obtained for different soils, crops and cropping systems across the countries (Ao et al., 2009; Escober et al., 1966; Tejada and Gonzalez, 2007; Gupta and Khan, 2009). In short as well as long-term, vinasses application improved aggregate formation and its stability across the soil orders (Hati et al., 2006; Cunha et al., 1987). Increased microbial population and their activities to its soil application caused five times more in decomposition rate than farm yard manure (FYM) that enhanced the production of more structural carbohydrate and humified materials (Patil et al., 1984; Nayamangra et al., 2001). Application of vinasse by 5 cm soil depth, increased the mean weight diameter of the soil aggregates and aggregate stability more than farmer recommendation (FYM + 100% NPK) in soybean-wheat cropping system on vertisols in India (Hati et al., 2007). In China, it is found that application of vinasse at the rate of 75 t/ha increased the macro aggregates over farmer practices and had more of larger size (>1 mm) aggregates with high amount of biodegradable carbon and nitrogen that has more significance to soil fertility (Jiang et al., 2012; Elliott, 1986). In short-term (<5 years), use of both treated and non-treated sugarcane vinasse application, significantly decreased bulk density and increased hydraulic conductivity of reclaimed sodic soils in India (Mahendra et al., 2010). Increased soil organic matter content from vinasse practices made changes in pore size distribution for capillary porosity and eventually increased the soil water content (Aggelides and Londra, 2000). Water retention is getting increased at field capacity state rather at permanent wilting point for increased micro pores in soils and it also reduced the soil penetration resistance (Webber, 1978; Weil and Kroontje, 1979). It increased soil humified materials at clay minerals interface and eventually soil external surface area for water and nutrient adsorption (Khaleel et al., 1981). Humified materials in vinasse binds the clay particles that had interfered in soil particles size content where the sand and silt size particles get increased over the clay content in the experiments conducted in China and Cuba (Rosabal et al., 2007; Jiang et al., 2012).

Raw vinasse application in soils, initially decreased soil pH for high content of free hydrogen ions but later stage of 30 or 45 days after the application increased to optimum level for organic matter oxidation (H^+ act as electron acceptor), soil saturation by exchangeable base, reduction of exchangeable Al, high buffering capacity of the clays, nominal presence of any weak salts namely carbonates or bicarbonates and release of free cations (Gemtos et al., 1999; Jiang et al., 2012; Hati et al., 2007; Aggelides and Londra, 2000). Increased total soil salt content as consequence of application is big concern for long-term disposal in agriculture (Madejon et al., 1995; Gemtos et al., 1995). However, the soil salinity content on post-harvest soil was not increased beyond the critical value of crops and rather it was observed only in laboratory condition (Gemtos et al., 1995). Short-term vinasse application on soil salinity on wetlands observed the minimum and well below to crop threshold value of many crops. It was due to regular irrigation practice that leached out the salt beyond the crops root zone (Rhoades and Loveday, 1990; Raverkar et al., 2000). For example in India, application of vinasse at increasing levels (10 to 40% Post metahnated spent wash) reported less increase of electrical conductivity (EC) in plough layers of wheat-rice cropping system than wheat-maize cropping system and in sugarcane field of Mauritius (Chhonkar et al., 2000; Jain et al., 2005).

For vinasse dose optimization, land basic character such as land slope, salinity level, soil permeability etc., and system approach rather single component for manure, cyclic and mixing mode of application than long-term continuous disposal in agriculture are need to be considered for long-term eco-friendly utilization (Chhonkar et al., 2000). Vinasses recommendation on the basis of soil permeability worked well in Brazil where sugarcane cultivation in normal soils and highly permeable soils have recommendation of 400 m^3/ha or below and 300 m^3/ha or lower, respectively (Pexioto and Coelho, 1981; Rodella and Ferrari, 1977). Application of vinasse by increasing rate (0, 150 and 300 m^3 ha^{-1} increased the variable net negative charges and decreased the value of zero point charge in oxisols than ultisols that moderated soil flocculation and dispersion behaviour (Musee et al., 2007). Das et al. (2010) reported for increasing trend of Ca, Mg, Na and K content for the spentwash ascending rate (40 to160 m^3/ha) application in red lateritic soils and it is indicative for resilient character and plant nutrition. In Spain, application of sugarbeet vinasse compost at moderate rate in calcareous sandy loamy soils has positive effect on soil organic matter, humic substance, Kjeldahl-N contents, cation exchange capacity and has residual effect to the subsequent crops (Madejon et al., 2001).

Fertilization of soil nitrogen in excess of crop demand tends to be lost by leaching, volatilization etc. and causes water pollution to eventually affect the environmental quality (Jackson and Smith, 1997; Thomsen, 2004). Dutil and Muller (1979) and Bustamante et al. (2010) reported from vinasse application for reduced available nitrogen at the beginning and 40% of the organic N was mineralized after 48 weeks to meet pace of the crop demand. For increased plant nitrogen utilization, application of vinasse recommended 1 month before to planting or sowing to avoid the potential leaching losses otherwise it would cause nitrate pollution in ground water (Delin and Engstrom, 2010). Rock phosphate and spent wash blended application (1:20) improved the phosphorous uptake and had residual effect to succeeding crops (Kumari and Phogat, 2012). This significant increase in soil available phosphorous is due to moderate content in spent wash and production of organic acids upon decomposition of vinasse in soils reduced the soil phosphorous fixation and increased the native phosphorous availability by solubilisation action on native phosphorous (Mahimairaja and Bolan, 2004). With high potassium content in vinasse, it is applied to meet out the 100% potassium requirement of wheat crop in Egypt (Arafat and Yassen, 2002). As ameliorating agents, treated spent wash application by 100 km/hac and 100% recommended dose of NPK regarded for highest exchange of Na by calcium that attributed from spent wash source (Deshpande et al., 2012). In India as per observation from Valliappan (1998), one time application of 150 m^3 ha^{-1} followed by two leaching and transplanting of rice on 40^{th} day is recommended practice for its cheaper and better in efficiency than gypsum application to sodic soils reclamation.

Soil microbial properties are influenced by pH, organic matter, C/N ratio, humidity and temperature (Stotzky and Norman, 1961). High organic matter and C:N ratio of vinasse materials mediated the microbial population and it is classified into two stages (Parnaudeau et al., 2008). One is carbonic stage where up to 45 days, the increased microbial population is due to oxidation of organic matter and beyond the days, the blooms would have been by mineralized nutrients. In Brazil, application of vinasse by 800 m^3 ha^{-1} as irrigation to sugarcane at 30 days interval found the fungi domination to first 15 days and later up to 45 days (Casarini et al., 1987). In contrast, domination of actinomycetes population over others was found in sugarcane field of China where it had been irrigated with vinasse of 120 m^3/ha as 1:5 ratio dilutions. It is concluded for advantage of soil microbial properties, where it is major component for nutrient cycling and sustainability of ecosystem (Flanagan and Cleve, 1983). Increase of microbial biomass C and N about 75% over farmer practices as well as increased microbial induced enzyme activities such as cellulase, aminopeptidase and phosphatase in China and also other countries are reported well (Shang-Dong et al., 2013; Su et al., 2009).

In India, split dose application of post methanated sugarcane spent wash about 187.5 m^3 ha^{-1} in sugarcane has increased the microbial population and enzymatic activities of urease, phosphatase and dehydrogenase (Selvamurugan et al., 2001). Observation of spent wash impact over nitrogen fixing bacteria paved the way for alternative mode of spent wash management (as 1:1 ratio) to pulses crops (Purushottam et al., 1986; Juwarkar and Dutta, 2003). However, over all the utilization of distillery spent wash alone or combination with other water materials (biocompost etc.) increased the activities of soil microbes and enzymes thus enhancing the soil fertility status besides an eco-friendly disposal. With these studies from different countries, it is concluded that appropriate application of vinasse with consideration of all local factors optimized for improved soil health of degraded lands sodic soils and crop production without polluting the environment (Chhonkar et al., 2000; Saliha, 2005). It is being considered as analogous to salty water for ionic composition, need to verify for cyclic and mixed mode approach on different cropping system with modern irrigation system for increased utilization in agriculture and better eco-friendly disposal in agriculture.

SIGNIFICANCE OF VINASSE UTILIZATION IN AGRICULTURE AND CROP QUALITY

Crop improvements are usually appreciated in terms of quality and quantity parameters; in vinasse it is about its nutritional value and optimization of soil properties to crops (Tejada and Gonzalez, 2006; Osman, 2010). Most of the countries have recommended vinasse application for high content of major nutrients to crops. Gomez (1996) gave recommendation to substitute 55% N, 72% P and 100% K to sugarcane crop in Venezuela. Similarly, vinasse irrigation of about 5 ml/L in sandy soil has substituted 62% P and 100% K to wheat crops in Egypt (Arafat and Yassen, 2002) and once again Gemtos et al. (1999) came out with recommendation to central part of Greece that vinasse by 7 t/ha for every 4 years can substitute for N requirement of wheat crops and given the significant yield increase over the farmer practices. As per meta-analysis of vinasse nutrition potential data, America can be able to derive the complete requirement of potassium and 68% phosphorus consumption for crop production. Absolute production and nutrient content of vinasse would have been to substitute significant fraction on nutrient consumption of countries particularly, America, Europe, China and India (Table 1).

Application of methanated sugarcane spent wash between 125 to 250 m^3 ha^{-1} has not affected the dry land crops rather it improved the germination, growth and yield of crops (Mahimairaja and Bolan, 2004). In Greece, vinasse practice 20 tha^{-1}, while sowing was not affected by the seed germination of cotton, bean and corn crops (Assimakopoulos, 2000). The vinasse application increased significantly N, P, K, S and Ca uptake as well as yield of sugarcane, wheat, pigeon pea and maize yield over the countries (Komdorfer and Anderson, 1993). Vegetable crops are sensitive to vinasse and irrigation by 33% dilution increased the fruit size and weight (Chidankumar et al., 2009). Application of spent wash along with rock phosphate (1:20) to fodder sorghum increased the phosphorous uptake by 30 and 26% on yield than sole rock phosphate or spent wash application (Kumari and Phogat, 2010). Application of raw spent wash at 50% dilution as irrigation in sugarcane has significance over plant height, length and girth of stem, leaves breadth, number of leaves and number of tillers per plant, leaf area index and total chlorophyll content than usual prevailing fertilizer recommendation in India (Rath et al., 2011). Application of vinnase of about 120 m^3/ha is considered as optimum recommendation to rice cultivation in low fertile red and lateritic soils where 25% of yield is increased over farmer practices (Das et al., 2010). In Mauritius, vinasse application of 100 m^3 ha^{-1} for 10 years to sugarcane crops produces additionally cane yield of 80 to 90 tonnes that is equivalent to one more crop season (Soobadar and KeeKwong, 2012). Vinasse practice of 80 m^3 ha^{-1} in entisols to sugarcane crops is better than trash management and farmers practice by yield, soil C and nitrogen stocks improvement (Alexander et al., 2006; Soobadarand and KeeKwong, 2012). Similarly, different mode of vinasse practices and significant increase in crops yield over major countries are given in Table 4.

Table 5. Kinds of stillage and their composition for animal feeds.

Particulars (%)	Wheat	Barley	Maize	Potato	Sorghum	Corn
Dry matter	12	28.9	6.2	6.0	5.8	Na
Crude protein	3.8	15.4	1.3	1.45	1.7	16.8
Fat	2.3	na	1.3	0.05	Nd	8.1
Crude fibre	0.12	na	0.1	0.7	1.51	Na
Sugars	6	na	2.8	3.1	2.6	Na
Starch	na	11.0	0.5	Nd	1.01	22
Ash	0.156	4.15	0.8	0.7	3.77	5.9

Adopted from Krzywonos et al. (2009)

STILLAGE SIGNIFICANCE ON ANIMAL QUALITY

Grains vinasses are called stillage, used as feeding materials to animals where these residues contain ¾th of non-starch component as broken grains and soluble. The soluble are concentrated to around 30 to 35% dry matter and used to feed the animals as such or along with coarse grains before the dehydration and they referred by wet distiller's grains (WDG), distiller's dried grains (DDG), distiller's dried soluble (DDS) and distillers dried grains with soluble (DDGS). It contains degraded yeast cells, soluble proteins rich in exogenous amino acids, large amounts of vitamin-B and minerals that are characterized by very high nutritive value to animals (Linda et al., 2005; Mustafa et al., 2000). These compositions are varied with kinds of grains used in the process and their original composition, fermentation process and their ethanol conversion efficiency (Table 5). In Australia, substantial proportion are recommended in diet ration to nursery pigs, boars, broilers, Layers, cattles and fishes as 25, 50, 10, 15, 20 and 5 - 20% of feeding materials, respectively (Bonnardeaux, 2007) and studies found the stillage significance over the diet digestibility and performance of the animals like normal feed (Gibb et al., 2008). It could be supplementary of water and feed materials to the ruminants in lean season of dry land regions where usually feed shortage experienced. Storability of WDG is increased by addition of corn or hay and lactic bacterial inoculants to WDG (Garcia and Kalscheur, 2004). By and large, stillage played significant role by improved animal survivability in the lean season, animal health and yield however, studies on technology development over processing vinasses materials for storage as well as nutrient enrichment is needed.

IMPLICATION OF VINASSE APPLICATION IN AGRICULTURE AND GROUND WATER QUALITY

As consequence of vinasse application in agriculture, the underground and surface water quality depends on land characters such as slope, soil depth, clay content and hydraulic properties and vinasse properties like chemical composition, time and rate of application and depth of ground water table (Jain et al., 2005). High EC, potassium, chloride, sulphate and melanoidine as colorant are potential components in vinasse to cause the pollution (Kumar and Gopal, 2001; Filho, 1996). Studies in many countries concluded for faulty management practices of vinasse caused the underground water pollution (Schoor, 2004). Indiscriminate application in agriculture to the areas of shallow water table (<15 m) associated with sandy soils of high infiltration rate are highly prone to pollution and that could not been recommended.

However, continuous application of vinasse 600 m^3/ha in sandy soils of sugarcane field could not cause the ground water pollution (Muhammad et al., 2012; Filho, 1996). Generalized rate of application in agriculture is normally not beyond the 500 m^3/ha and it has meagre probability for ground water pollution due to their absorptivity and microbial mediated oxidation of vinasse, filter mechanism of soils and low mobility rate constants of nitrate and other major anions in the soils (Allred et al., 2007; Karanam and Joshi, 2010). In Brazil, continuous application of vinasse about 300 m^3/ha in clay loam soils for 15 years have not altered the ground water quality and leach out studies found the decreased amounts of nitrate and other pollutant at water front with depth where rate of anions mobility in soils are very low (Allred et al., 2007; Cruz et al., 1991). It is well supported by studies in India where field irrigation by 40% of treated spent wash application to sandy loam soils (Ustrochrept) found decreased spent wash concentration and chloride with soils depth (Karanam, 2001). Application at rainy seasons particularly areas near moderate sloppy lands are highly subject to surface run-off and leaching that pollute the flowing waters and these are against the normal recommendation (Shenbagavalli et al., 2011). Over the countries, ground water near industrial areas are highly polluted for continuous storage in lagoon ponds that leached into water tables and therefore need felt amendments was made for structural modification (Chaudhary and Mahima, 2011). For continuous monitoring of water quality in the sensitive areas of degraded soils where vinasse have been practiced as irrigation or basal application in agriculture (salt affected soils) have recommended 15 m length permanent pores in four numbers per 10 hac (EPA, 2012). In water quality aspect, India already made safety measures and given the following recommendation: (1) For ferti-irrigation, waste water is required to be treated up to BOD of 100 mg/L and total dissolved solids (TDS) of 2100 mg/L, (2) Fertigation, composting and land application options should not be taken up in rainy season (CPCB, 2008). Indiscriminate direct disposal of waste water from distilleries to water bodies of Ganga and Gomati River in India caused the dissolved oxygen content less than 2.25 mg/L and lethal concentration 50 (LC_{50}) value of 0.5% are critical for fingerling mortalities (Joshi, 1988). Approach of ground water monitoring, optimized time and space application with mixing of good quality water and judicious application of vinasse in agriculture need to be considered against ensured water quality (Chhonkar et al., 2000). Therefore, appropriate time and space in judicious application of vinasse in agriculture is not merely environmental friendly way of utilization, but might be a component to enhance the environmental quality.

Final consideration

Appropriate time, space and rate of vinasse application in agriculture has added significant amount of nutrients, improved the soil quality of degraded land and increased of crop yields. Crop yield increment is varied for soil fertility status, spent wash composition, methods of application and inherent ability of crops. Starch based vinnase material used as ration in animal feeds increased animal quality and survival ability in the off-season period. However, water quality of many parts of the world affected severely by indiscriminate and inappropriate application and storage for treatments in surrounding areas of distillery industries possess biggest challenge in handling of increased production of spent wash.

Conflict of Interest

The authors have not declared any conflict of interest.

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Molecular studies on transmission of mung bean yellow mosaic virus (MYMV) by *Bemisia tabaci* Genn. in Mungbean

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The whitefly *Bemisia tabaci* Genn. is an important pest worldwide because of its ability to cause damage by direct feeding and its role as a vector of plant viruses including geminiviruses. Yellow mosaic virus (MYV) is a serious disease of pulse crops including mungbean, blackgram, frenchbean, pigeonpea and soybean. Yellow mosaic diseases are one of the most important viral diseases in mungbean caused by mungbean yellow mosaic virus (MYMV) which lead to severe yield reduction and it necessitates development of MYMV resistant lines for improved crop yield. Basic studies were carried out to elucidate the characteristics of MYMV transmission by its vector, *B. tabaci*. Artificial transmission experiments with *B. tabaci* were conducted under greenhouse conditions using cylindrical nylon cages with wire mesh tops. After 24 h acquisition access period (ASP) on agroinfected mungbean plants, *B. tabaci* collected from these agroinfected mungbean plants were considered viruliferous and transferred to a separate cage with healthy mungbean plants as confirmed via agroinoculation. After 24 h inoculation access period (IAP), *B. tabaci* were removed and the plants were sprayed with an insecticide and kept for observations of symptom development for 10 to 25 days in insect cages. Studies concluded with mungbean accessions using ten whitefly adults with 24 h of ASP and IAP resulted in transmission of virus of 70.50, and percent in MYMVR 111 (*At* VA 221), MYMVR 29 (*At* VA 239) and MYMVR 29 (*At* VA 221) respectively. Ten viruliferous whitefly adults did not cause MYMV symptom in KMG 189 (*At* VA 221), ML818 (*At* VA 239) and MYMVR 57 (*At* VA 221). Twenty viruliferous whitefly adults were able to cause MYMV after 48 h ASP and 24 h IAP and resulted in the maximum transmission efficiency in MYMVR 55 (*At* VA 221) (85.00%) and MYMVR 55 (*At* VA 239) (83.50%). The virus was proven to be a persistent discrete fragment of 703 bp using the polymerase chain reaction method on viruliferous whitefly adults, while no bands were obtained from non-viruliferous *B. tabaci* adults reared on CO_2 brinjal host.

Key words: Mungbean yellow mosaic virus (MYMV), transmission, vector, *Bemisia tabaci*.

INTRODUCTION

The whitefly, *Bemisia tabaci* (Genn.) is one of the most economically important pests in many tropical and subtropical regions (Bock, 1982). This polyphagous pest can cause extensive damage in more than 500 species of agricultural and horticultural crops (Greathead, 1986) through its direct feeding, and its ability to directly transmit geminiviruses. Mungbean (*Vigna radiata* L.) is an important pulse crop in developing countries of Asia,

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Africa and Latin America where it is consumed as dry seeds or fresh green pods (Karuppanapandian et al., 2006). Mungbean serve as a vital source of vegetable protein (19 to 28%), mineral (0.18 to 0.21%) and vitamins. India is the leading mungbean producer, covering up to 55% of the total world acreage and 45% of total production (Rishi, 2009). Mungbean yellow mosaic virus belongs to family Geminiviridae (Fauquet et al., 2003). The family Geminiviridae is divided in to four genera, Mastrevirus, Curtovirus, Topocuvirus and Begomovirus (Ramos et al., 2008). Begomovirus is the largest genus of the family Geminiviridae (Dhakar et al., 2010) which is characterized by a pipartite genome or monopartite genomes that were transmitted in a circulative persistent manner by B. tabaci. Among biotic agents, plant viruses are responsible for a significant proportion of crop diseases (Prajapat et al., 2011). It causes serious economic losses in many major crops by reducing seed yield and quality (Kang et al., 2005). Yellow mosaic disease (YMD) is reported to be the most destructive viral disease, caused by yellow mosaic virus. Mungbean yellow mosaic virus causes severe yield reduction in all mungbean growing countries in Asia including India (Biswass et al., 2008). Among the various diseases, MYMV disease was given special attention because of its severity and ability to cause yield loss of up to 85% (AVRDC, 1998). Conventional methods are unsuccessful in developing MYMV resistant mungbean lines due to the lack of a reliable screening technique. Rogers et al., (1986) developed an innovative technique called “Agroinfection” which serve as an alternate route for viral infection of plants by using the Ti plasmid and was demonstrated in case of Tomato Golden Mosaic Virus. A new technique called agroinoculation was has been shown to be used successfully in screening. Agroinoculation was done using the viral constructs mobilized in *Agrobacterium tumefaciens* strains. This paper reports the molecular studies on transmission of Mungbean yellow mosaic virus (MYMV) by *B. tabaci* in mungbean and the development of a polymerase chain reaction-based technique to detect the virus from its insect vector.

MATERIALS AND METHODS

Mass culturing of *B. tabaci*

Field collected *B. tabaci* nymphs and adults were reared in insect cages containing thirty day old CO_2 brinjal plants to maintain a laboratory culture at the temperature 27 and relative humidity 70% for the studies three generation maintained and adult age two days after emergence and used for the experiments. *B. tabaci* needed for the insect transmission experiment were collected from the culture using an aspirator.

Acquisition access period of *B. tabaci* on MYMV agroinfected mungbean plants

Adult *B. tabaci* were collected from the laboratory culture with the help of an aspirator and transferred into a test tube which was then covered with muslin cloth. Adult whiteflies were starved for 2 h under cool conditions temperature at (24°C) at which time the mouth of the test tube was opened to allow the adults to transfer to MYMV agroinfected mungbean plants. Adult whiteflies 10 and 20 where then allowed to feed for an acquisition period of 24 and 48 h.

Agroinoculated mungbean plants

MYMV resistant agroinoculated mungbean plants were grown in pots and maintained in a greenhouse for future use in the transmission experiment. Need to describe conditions in the greenhouse (26°C and relative humidity 64.15%), media used to grow the plants, source of seed, watering regime, pot size. plant age at testing etc.

Insect (vector) transmission

The insect (vector) transmission protocol developed by Aidawati et al. (2002) was used. MYMV transmission experiments with *B. tabaci* were conducted using cylindrical nylon cages with mesh tops. Ten *B. tabaci* adults were introduced into the cage through a hole which was sealed afterwards. After 24 and 48 h acquisition access period, *B. tabaci* adults were removed from MYMV agroinfected mungbean plants and transferred to a separate cage containing healthy agro inoculated (resistant) mungbean plants. After 24 h inoculated access period *B. tabaci* adults were removed and the plants were sprayed with an insecticide (Dimethoate 30EC at 1 ml/L) and evaluated for MYMV symptom development 10-20 later. Five potted plants were used for each acquisition-access period and the percentage of virus infection was calculated from plants showing MYMV symptoms after 10-20 days. Resistance levels were assessed by visual scoring of symptoms under greenhouse conditions (26°C and relative humidity 64.15%), following the 1-9 grade scale for visual scoring of mungbean yellow mosaic virus diseases by Nene et al. (1981) (Table 1).

DNA extraction

Total nucleic acids were extracted from individual of viruliferous and non viruliferous whiteflies using CTAB (hexadecyl trimethyl ammonium bromide) method with necessary modifications. Quality and quantity of the isolated DNA was measured in Nanodrop® ND-1000 spectrophotometer (nanodrop technologies, USA) and 1.0% Agarose gel electrophoresis before being used as the template DNA for all polymerase chain reactions (PCR). The reagents were purchased from Bangalore Genei Ltd., Bangalore, India.

Detection of MYMV in *B. tabaci* by polymerase chain reaction

Adults of *B. tabaci* were collected after a 24 h acquisition access period. Sets of 10, 15, 20, 25 and 10 non-viruliferous *B. tabaci* were subjected to DNA extraction following the CTAB (hexadecyl trimethyl ammonium bromide) method of Goodwin et al. (1994), while DNA extraction from MYMV infected mungbean leaves was conducted using the method of Karuppanapandian et al. (2006). The method of Rojas et al. (1993) was used for amplification of viral DNA from *B. tabaci* extracts by polymerase chain reaction (PCR). The viruliferous nature of these insects were confirmed by polymerase chain reaction products amplified by viral coat protein gene specific primers. Individual insects of viruliferous *B. tabaci* in groups of 10, 15, 20, 25 and 10 non-viruliferous *B. tabaci*, MYMV infected mungbean leaf samples were taken for DNA extraction

Table 1. Grade scale for visual scoring MYMV diseases.

Grade	Description
1.	No visible symptom on leaves or very minute yellow specks on leaves
2.	Small yellow specks with restricted spread covering leaf area 0.1 to 5.0 %
3.	Yellow mottling of leaves covering leaf area 5.1 to 10%
4.	Yellow mottling of leaves covering leaf area 10.1 to 15%
5.	Yellow mottling and discoloration covering 15.1 to 30%
6.	Yellow mottling and discoloration covering 30.1 to 50%
7.	Pronounced yellow mottling and discoloration of leaves stunting of plants covering 50.1 to 75 %
8.	Severe yellow discoloration of leaves covering 75.1 to 90%
9.	Very severe yellow discoloration of leaves covering 90.1 to 100%

Table 2. Transmission efficiency of Mung bean yellow mosaic virus (MYMV) by *Bemisia tabaci* in Mungbean.

Name of the agroinoculated mungbean lines	Number of insects released per plant	Acquisition feeding period (Hours)	Inoculation period (Hours)	No. of days taken for symptom development	MYMV Infectivity (%)
KMG 189 (*At* VA 221)	10	24	24	-	No
ML818 (*At* VA 239)	10	24	24	-	No
ML 1108 (*At* VA 221)	10	24	24	-	No
MYMVR 29 (*At* VA 221)	10	24	24	16	65.00
MYMVR 29 (*At* VA 239)	10	24	24	16	69.00
MYMVR 57 (*At* VA 221)	10	24	24	-	No
MYMVR 95 (*At* VA 221)	10	24	24	-	No
MYMVR 95 (*At* VA 239)	10	24	24	-	No
MYMVR28 (*At* VA 221)	10	24	24	-	No
MYMVR28 (*At* VA 239)	10	24	24	-	No
MYMVR 111 (*At* VA 221)	10	24	24	15	70.50
SP 84 (VA221)	10	24	24	-	No
MYMVR 115 (*At* VA 221)	10	24	24	15	55.00
MYMVR 115 (*At* VA 239)	10	24	24	16	60.00
MYMVR 55 (*At* VA 221)	10	24	24	16	54.00
MYMVR 55 (*At* VA 239)	10	24	24	16	50.00
MYMVR80 (*At* VA 221)	10	24	24	-	No
MYMVR80 (*At* VA 239)	10	24	24	-	No
MYMVR 90 (*At* VA 239)	10	24	24	15	53.00
MYMVR90 (*At* VA 221)	10	24	24	-	No

NO - indicate that the plants did not show any visible symptom during observation period.

followed by PCR amplification along with DNA clone of *A. tumefaciens.* Amplified DNA fragments were electrophoresed in 1% agarose minigels in TBE buffer and detected with UV light after staining in ethidium bromide (Maniatis et al., 1982).

RESULTS

The results revealed that transmission experiment after a 24 h acquisition access and 24 h inoculation assess period ten *B. tabaci* viruliferous adults were able to cause transmission of virus up to 70.50, 69.00, 65.00, 60.00. 55.00, 54.00 and 53.00% in MYMVR 111 (*At* VA 221), MYMVR 29 (*At* VA 239), MYMVR 29 (*At* VA 221), MYMVR 115 (*At* VA 239), MYMVR 115 (*At* VA 221), MYMVR 55 (*At* VA 221) and MYMVR 90 (*At* VA 239), respectively (Table 2). Ten adults of viruliferous adults of *B. tabaci* did not cause MYMV symptom in KMG 189 (*At* VA 221), ML818 (*At* VA 239), MYMVR 57 (*At* VA 221), MYMVR 95 (*At* VA 221), MYMVR 95, MYMVR28 (*At* VA 221), MYMVR28 (*At* VA 239), MYMVR80 (*At* VA 221), MYMVR80 (*At* VA 239) and MYMVR90 (*At* VA 221). The control plants inoculated with non-viruliferous whiteflies did not show MYMV symptoms.

The results (Table 3) indicated that twenty viruliferous adults of *B. tabaci* did not cause YMV symptom after 48 h acquisition access and 24 inoculation assess period in KMG 189 (*At* VA 221), ML818 (*At* VA 239), ML 1108 (*At* VA 221), MYMVR 57 (*At* VA 221), MYMVR 95 (*At* VA 221), MYMVR 95 (*At* VA 239), MYMVR28 (*At* VA 221), MYMVR28 (*At* VA 239) SP 84 (VA221), MYMVR80 (*At* VA 221) and MYMVR80 (*At* VA 239). One entry namely MYMVR90 showed resistance against *At* VA 221 strain

Table 3. Transmission efficiency of Mung bean yellow mosaic virus (MYMV) by *B. tabaci* in Mungbean.

Name of the agroinoculated mungbean lines	Number of insects released per plant	Acquisition feeding period (Hours)	Inoculation period (Hours)	No. of days taken for symptom development	MYMV Infectivity (%)
KMG 189 (*At* VA 221)	20	48	24	-	No
ML818 (*At* VA 239)	20	48	24	-	No
ML 1108 (*At* VA 221)	20	48	24	-	No
MYMVR 29 (*At* VA 221)	20	48	24	15	75.00
MYMVR 29 (*At* VA 239)	20	48	24	16	70.00
MYMVR 57 (*At* VA 221)	20	48	24	-	No
MYMVR 95 (*At* VA 221)	20	48	24	-	No
MYMVR 95 (*At* VA 239)	20	48	24	-	No
MYMVR28 (*At* VA 221)	20	48	24	-	No
MYMVR28 (*At* VA 239)	20	48	24	-	No
MYMVR 111 (*At* VA 221)	20	48	24	16	80.50
SP 84 (VA221)	20	48	24	-	No
MYMVR 115 (*At* VA 221)	20	48	24	15	75.00
MYMVR 115 (*At* VA 239)	20	48	24	15	81.00
MYMVR 55 (*At* VA 221)	20	48	24	16	85.00
MYMVR 55 (*At* VA 239)	20	48	24	15	83.50
MYMVR80 (*At* VA 221)	20	48	24	-	No
MYMVR80 (*At* VA 239)	20	48	24	-	No
MYMVR 90 (*At* VA 239)	10	48	24	15	83.00
MYMVR90 (*At* VA 221)	20	48	24	-	No

NO - indicate that the plants did not show any visible symptom during observation period.

and it was found to be susceptible to *At* VA 239 strain. Twenty viruliferous adults of *B. tabaci* were able to cause MYMV after 48 h acquisition and 24 h inoculation assess period resulted the maximum transmission efficiency in MYMVR 55 (*At* VA 221) (85.00%), which was followed by MYMVR 55 (*At* VA 239) (83.50%), MYMVR 90 (*At* VA 239) (83.00%), MYMVR 29 (*At* VA 221) (75.00%) and MYMVR 29 (*At* VA 239) (70.00%). Twenty viruliferous adults of *B. tabaci* were did not cause MYMV symptom in KMG 189 (*At* VA 221), ML818 (*At* VA 239), MYMVR 57 (*At* VA 221), MYMVR 95 (*At* VA 221), MYMVR 95, MYMVR28 (*At* VA 221) and MYMVR28 (*At* VA 239), MYMVR80 (*At* VA 221), MYMVR80 (*At* VA 239) and MYMVR90 (*At* VA 221) after 48 h acquisition and 24 h inoculation assess period. Twenty whiteflies per plants were found to be effective for disease transmission. Typical symptoms appeared after a minimum incubation period of 24 h under green house condition. The control plants inoculated with non-viruliferous whiteflies did not show MYMV symptoms. The characteristics MYMV symptoms observed on naturally infected plants were appear in the form of small irregular yellow specs and spots along the veins, which enlarge until leaves were completely yellowed fewer flowers and pods that bear smaller, occasionally shriveled seeds in severe cases MYMV symptoms observed after 15 - 16 days of after virus inoculation.

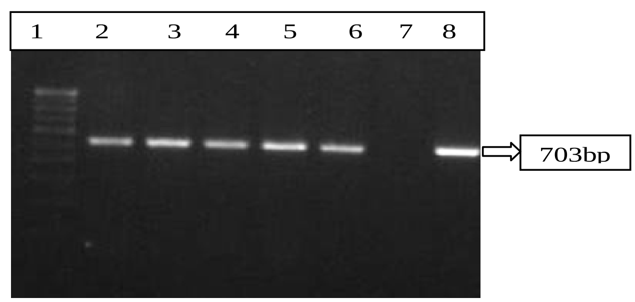

Figure 1. Polymerase chain reaction-amplified product of MYMV from viruliferous *B. tabaci*. Amplification of 703bp fragments with the primer pair coat protein forward and reverse primer (gene specific primers). A 15 µl aliquot of each polymerase chain reaction mixture was analyzed in a 1.0% agarose gel. Lane 1 - 100 bp ladder size marker. The samples are Lane 2) 10 individual of viruliferous *B. tabaci* with MYMV, Lane 3) 15 individual of viruliferous *B. tabaci* with MYMV, Lane 4) 20 individual of viruliferous *B. tabaci* with MYMV, Lane 5) 25 individual of viruliferous *B. tabaci* with MYMV, Lane 6) MYMV - infected mung bean, Lane 7) 10 individual of non viruliferous *B. tabaci*, Lane 8) DNA clone of *At* VA 239.

Detection of MYMV in *B. tabaci* by polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) amplified fragments of the predicted size from the annealing positions of the coat protein (gene specific) primers were obtained from groups (10, 15, 20 and 25) of viruliferous *B. tabaci* and 10 non viruliferous *B. tabaci* (Figure 1). The virus was proven to be persistently discrete fragments of 703 bp were observed when polymerase chain reaction method was applied to detect the virus in viruliferous adults of *B. tabaci*, while no bands were obtained from non-viruliferous *B. tabaci* adults.

DISCUSSION

Geminiviruses are single-stranded DNA plant viruses with one or two circular genome components of 2.7 to 3.0 kb in size, encapsidated in twinned particles. They are transmitted by whiteflies. The whitefly species *B. tabaci* is the most efficient vector of members of the genus Begomovirus (1998; Van Regenmortel et al., 2000). Begomoviruses are currently emerging as a major threat in many tropical and subtropical regions in worldwide (Varma and Malathi, 2003). The probability of subsequent transmission of circulative viruses by insect vectors generally increases with increasing acquisition access period until all insects that are able to do so have acquired the virus (Swenson, 1967). Virus acquisition by insect vectors may depend on the virus titer in the infected plant, the ability of the insect to ingest the virus, and the passage of the virus through the midgut wall and subsequent survival in the insect vector. Transmission of MYMV was observed with ten adults of *B. tabaci* were able to cause transmission efficiency of virus up to 70.50, 69.00, 65.00, 60.00. 55.00, 54.00 and 53.00% in MYMVR 111 (*At* VA 221), MYMVR 29 (*At* VA 239), MYMVR 29 (*At* VA 221), MYMVR 115 (*At* VA 239), MYMVR 115 (*At* VA 221), MYMVR 55 (*At* VA 221) and MYMVR 90 (*At* VA 239), respectively (Table 2). The results reported by Aidawati et al. (2002) 100% tobacco leaf curl virus transmission efficiency occurred with a 24 h acquisition access period and inoculation access period. The results reported by Mehta et al. (1994) 24 h acquisition and inoculation access period was achieved maximum transmission efficiency with tomato yellow leaf curl virus.

After 48 h acquisition access period and 24 h inoculation access period resulted maximum transmission efficiency observed in MYMVR 55 (*At* VA 221), MYMVR 55 (*At* VA 239), MYMVR 90 (*At* VA 239), MYMVR 111 (*At* VA 221), MYMVR 29 (*At* VA 221) and ML 1108 (*At* VA 221) in 85.00, 83.50, 83.00, 81.00, 80.50, 75.00 and 70.00%, respectively (Table 3). Aidawati et al. (2002) reported that twenty *B. tabaci* adults cause 100% tobacco leaf curl virus transmission efficiency occurred with a 24 h acquisition access and inoculation assess period in tobacco. Transmission of begomoviruses from Indonesia by *B. tabaci* has been demonstrated earlier (Aidawati et al., 2002; Sudiono et al., 2001; Rusli et al., 1999).

The expected fragment of viral DNA 703 bp was amplified from a ten adult of viruliferous *B. tabaci* while no bands were obtained from non-viruliferous *B. tabaci* adults (Figure 1). Detection of MYMV from viruliferous *B. tabaci* using PCR technique showed that amount of viral DNA amplified in the polymerase chain reaction became shown higher as shown by the brightness of the DNA fragment in the gel electrophoresis. The results reported by Aidawati et al. (2002) tobacco leaf curl viral DNA fragments of 1.6 kb were observed when polymerase chain reaction method was applied to detect the virus in viruliferous nymphs and individual adults of *B. tabaci*, while no bands were obtained from non-viruliferous adults. The results were shown by Butter and Rataul (1977) with tomato leaf curl virus, as well as, Cohen and Nitzany (1966) and Mehta et al. (1994) with tomato yellow leaf curl virus. The virus persist inside the host genome the symptom development of 1-16 days after inoculation. The results reported by Cohen and Nitzany (1966) persistency of virus in the insect body varies, for example, 1-15 days for tomato yellow leaf curl virus, 8-55 days for tomato leaf curl virus (Butter and Rattaul, 1977).

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Response of durum wheat varieties to water in semi-arid Algeria

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Irrigation and varietal improvement are two major ways of increasing and stabilizing durum wheat (*Triticum durum* Desf.) production in semi-arid Mediterranean countries. A 3-year study was conducted in Khemis-Miliana (Upper Chelif, Algeria) to evaluate the yield response of six durum wheat genotypes to deficit irrigation. Grain yield in the unirrigated treatment ranged from 2.0 $t.ha^{-1}$ (2008) to 2.8 $t.ha^{-1}$ (2009). In rainfed conditions, the local variety Mohammed Ben Bachir (MBB) was the least productive (yield < 2.0 $t.ha^{-1}$) but the most stable, being the most insensitive to early drought. Yields of Bousselem exceeded 3.0 $t.ha^{-1}$ whereas Mexicali and Vitron had more variable and lower yields. Rainwater productivity (RWPg, the ratio of grain yield to precipitation) ranged from 0.5 (MBB) to 1.1 $kg.m^{-3}$ for Bousselem, Chen's, and Waha, three varieties known to be drought-tolerant. Deficit irrigation (140 mm) resulted in an increase in grain yield of 0.4 to 3.2 $t.ha^{-1}$ depending on weather conditions and variety, with a mean response of 1.1 $t.ha^{-1}$. Irrigating between shooting and booting increased straw production by 23% and grain production by 46% on average. The most explanatory components of final yield under rainfed management were the number of $ears.m^{-2}$ and the number of grains per ear, while under irrigated management the number of grains per ear and the thousand grain weight were more critical for yield determination. The development of irrigation on durum wheat could help to close the gap between current and attainable grain yields in semi-arid Algeria, provided groundwater is available and the flowering period escapes desiccating hot winds. Wheat breeding should be focused on developing genotypes with stable behavior under drought but which respond well to irrigation.

Key words: Irrigation, drought, yield components.

INTRODUCTION

In Algeria, durum wheat (*Triticum durum* Desf.), grown on 47% of the cultivated area, constitutes the main small-grain cereal (Haddouche and Mekliche, 2008). Since the 70s cereal production has failed to meet the needs of the population. As a result, the country imports between 1 and 2 Mt of durum wheat a year as the staple to make

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bread and couscous (Smadhi and Zella, 2009). Although the area under cereal production fluctuates around 3 million hectares (FAOSTAT, 2014), 60% of this area has a semi-arid climate in regions where the farmers traditionally use a limited amount of inputs (seeds, fertilizers), resulting in a low attainable yield level. Effectively, in those areas where annual rainfall is below 450 mm on average and falling largely in winter, durum wheat yield ranges from 0.7 to 1 $t.ha^{-1}$ and in future the year-to-year variability in Algeria may become more extreme due to rainfall patterns becoming more erratic and unpredictable (Feliachi et al, 2001; Smadhi and Zella, 2009; Sahnoune et al., 2013).

It has been well established that drought is the major limiting factor of wheat yield in north Africa, the losses ranging from 10 to 80% of potential yield depending on the year (Nachit et al., 1998). In this environment, once the soil water present at planting in November has been exhausted, the amount of rainfall received in the spring determines the level of attainable yield in the absence of supplementary irrigation (Chennafi et al., 2006). Although water stress may occur at any time during crop life, terminal heat and drought stress are the rule (Baldy, 1993). However, one feature of the climate is also uncertain rainfall in the early stages of the winter wheat crop (El Hafid et al., 1998a). The effects on crop development, growth and grain yield depend on the timing and intensity of water stress (Mogensen et al., 1985; Musick and Porter, 1990; Debaeke et al., 1996). Thus, if drought occurs during the two weeks before heading, it can reduce the number of grains per spikelet (Fisher, 1973) while the lack of water at the end of the season reduces the individual grain weight (Kobata et al., 1992). The number of ears per m² will be reduced by drought occurring from crop tillering (Assem et al., 2006).

To fill the gap between domestic needs and national cereal production, Algeria needs to quadruple its local production either by increasing the sown area from 3 to 12 million hectares (that is, by reducing the area of fallow land) or by raising the average yield from 0.7 to 2.8 $t.ha^{-1}$ (Smadhi and Zella, 2009). This highlights the huge technical progress required. To increase and stabilize grain yield at this level, improved genotypes combined with appropriate crop management are strongly recommended (Bouthiba et al., 2008).

Improving drought tolerance of winter cereals has long been the main target for breeders in the Mediterranean region but substituting the local landraces by varieties selected for high yield potential is also an objective (Monneveux and Ben Salem, 1992; Rajaram and Hettel, 1994; Nachit et al., 1998; Hafsi et al., 2001; Richards et al., 2002). A relatively small number of durum varieties are grown in Algeria, either local or recently introduced (Benbelkacem and Kellou, 2001). Local genotypes are characterized by a low but relatively stable yield potential. Conversely, introduced varieties can give a high yield but only under favorable conditions of water supply and temperature. Adoption of short-term varieties was intended in semi-arid Algeria for an effective use of limited soil water and to reduce the effects of terminal stress, but grain yield remains very low anyway (Annicchiarico et al., 2006).

Therefore, to achieve this potential and stabilize production in semi-arid Mediterranean regions, supplemental irrigation of wheat has been proposed in addition to varietal choice and zero tillage (Bouthiba et al., 2008; Karrou, 2013). However, faced with the scarcity of water resources, exacerbated by possible climate change, the use of irrigation has to be optimized and adapted to genotype and wheat crop management.

The generic response of durum wheat to irrigation has been studied extensively in north Africa and west Asia (Oweis et al., 1999; Zhang and Oweis, 1999; Chennafi et al., 2006; Oweis and Hachum, 2006; Bouthiba et al., 2008; Karam et al., 2009). However very few studies have compared the differential response (in terms of yield and water use efficiency) of a range of wheat genotypes to several water regimes (Bouthiba et al., 2008; Mohammadi et al., 2011). However, it is foreseeable that interactions between water availability and plant response may occur depending on the level of drought tolerance of a variety and its growth pattern.

To assess the significance of these interactions, a 3-year experimental study was conducted in semi-arid conditions of Algeria, involving a range of local and introduced genotypes and different water stress levels varying in timing and intensity. This range of constraints was achieved by combining different irrigation and rainfall patterns that were more or less deficient in terms of crop water requirements. The main objective of this study was to analyze the yield response of a range of durum wheat genotypes to deficit irrigation compared to rainfed management.

In cereal-livestock farming systems of north Africa and west Asia, the straw of durum wheat is frequently used for feeding animals during the dry season and may enhance the sustainability and the flexibility of farming in various respects (Annicchiarico et al., 2005). Therefore, our analysis will be extended to grain and straw response to genotype and irrigation.

MATERIALS AND METHODS

Permanent characteristics of the experimental site

The experimental site in Khemis-Miliana belongs to the 'Institut Technique des Grandes Cultures' (ITGC). It is located in the Upper Chelif, west of Algiers (36° 15'N, 02° 14'E, altitude 382 m) and is subjected to a semi-arid climate characterized by an average annual rainfall of 373 mm that occurs mostly in winter. This amount (recorded between November and February) accounts for 65% of the precipitation falling during the growing season.

A weather station on the experimental site provided the basic daily climatic data (Table 1): maximum and minimum temperatures, sunshine duration, wind speed, relative humidity, and precipitation. Over the period 1990 to 2008, cumulative rainfall from October to June averaged 351 mm; the corresponding ETo (Penman) was 737 mm, and the rainfall deficit (P-ETo) was 386 mm.

Table 1. Monthly precipitation (P, mm), Penman potential evapotranspiration (Eo, mm) and mean air temperature (°C) for the 3 growing seasons : 2007, 2008 and 2009.

Year	Parameters	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total Oct - Jun
1990 - 2009	Precipitation (mm)	33	54	57	56	46	44	38	25	6	359
	ETo (mm)	83	39	23	26	41	78	103	153	194	740
2006 - 2007	Precipitation (mm)	2	12	69	15	48	127	78	1	0	352
	ETo (mm)	97	48	21	26	45	70	85	150	194	736
	Mean temperature (°C)	22.8	17	11.4	10.5	13.3	11.6	16	20	25	16.4
2007 - 2008	Precipitation (mm)	58	110	39	26	18	63	7	25	8	354
	ETo (mm)	75	32	24	26	49	80	118	127	137	668
	Mean temperature (°C)	19.2	12.8	10.4	10.4	12.2	12.8	16.3	19.9	24.3	15.4
2008 - 2009	Precipitation (mm)	43	77	105	89	32	73	75	19	0	513
	ETo (mm)	76	40	21	32	47	89	96	166	205	772
	Mean temperature (°C)	20.6	13.1	9.9	10.2	10.9	14.2	14.4	23.4	28.2	16.1

Table 2. Main soil characteristics in Khemis-Miliana experimental station.

Soil characteristics	Soil layers (cm)			
	0-25	25-45	45-70	70-100
Clay (%)	30.3	31.0	30.5	43.0
Fine silt (%)	24.1	25.2	24.3	20.2
Silt (%)	24.4	24.7	24.5	18.8
Fine sand (%)	10.3	9.8	10.0	6.0
Sand (%)	10.9	9.3	10.7	12.0
Organic matter (%)	2.0	1.3	1.3	1.3
$CaCO_3$ (%)	7.7	7.7	7.3	10.4
pH	7.7	7.5	7.6	8.1
EC ($dS.m^{-1}$)	0.31	0.37	0.28	0.57
Bulk density	1.34	1.40	1.37	1.48
Soil moisture at field capacity (Pf 2.5), (%)	25.2	24.6	24.8	25.4
Soil moisture at wilting point (Pf 4.2), (%)	11.9	11.8	11.9	12.0

The physical and chemical soil properties are summarized in Table 2. Soil characteristics were determined by the Soil Science Department of the 'Ecole Nationale Supérieure d'Agronomie' in Algiers, using routine methods. The soil is a chalky silty clay. As its electrical conductivity (EC) is less than 4 $mmhos.cm^{-1}$, it is not considered as a saline soil. The cation exchange capacity (CEC) is high, and sodium represents less than 10% of the total CEC. Soil organic matter in the top layer is at 2%, but is very poor at depth. The available soil water content (ASWC) to 1 m depth is 183 mm. The saturated hydraulic conductivity is 1.2 cm h^{-1} (Ollier and Poirée, 1981).

The quality of irrigation water was assessed by the following variables: Ca^{++} (6.93 $meq.l^{-1}$), Mg^{++} (5.67 $meq.l^{-1}$), Na^{+} (6.43 $meq.l^{-1}$), HCO_3^{-} (3.75 $meq.l^{-1}$), EC (2.5 $dS.m^{-1}$) and pH (7.5). SAR (Sodium Adsorption Ratio) was 2.62, which presents no risk for soil degradation. Although this water salinity level is considered to be high, it should not affect the yield potential of durum wheat (Ayers and Westcot, 1985; Bauder et al., 2007).

Treatments and experimental design

Three field experiments comparing the response of six durum wheat varieties to irrigation were conducted between 2007 and 2009 to take advantage of the natural high variability of rainfall. The experimental layout was a split-plot design with irrigation as the main plot treatment (irrigated vs non irrigated) and the varieties as sub-plots, each being replicated three times within each main plot. The area of each basic plot was 6 m². The crop was planted in 6 rows, 5 m long, with 20 cm between rows.

Plant material

Six durum wheat genotypes were selected for this study: Bousselem (B), Chen's (C), Mohammed Ben Bachir (MBB), Mexicali (M), Vitron (V), and Waha (W). They are among the genotypes most commonly grown in Algeria (Annicchiarico et al., 2006).

Table 3. Main aspects of wheat crop management.

Year	Sowing date	Harvest date	Water regimes	Irrigation amounts (mm)				
				Tillering	Shooting	Heading	Maturation	Total
2007	17/12/06	08/06/07	Rainfed	0	0	0	0	0
			Irrigated	50	0	0	0	50
2008	12/12/07	08/06/08	Rainfed	0	0	0	0	0
			Irrigated	50	0	50	80	180
2009	28/12/08	07/06/09	Rainfed	0	0	0	0	0
			Irrigated	50	0	50	80	180

Only MBB is derived from breeding in a local population. Other varieties are accessions from CIMMYT (Chen's, Mexicali), ICARDA (Bousselem, Waha) or Spain (Vitron). In terms of earliness at heading, the varieties rank from the earliest to the latest as follows: C = M = W>V>B>MBB. The difference in maturity between C, M, W group and MBB is 10 days at heading (Mekhlouf et al., 2006).

Crop management

After an application of 69 kg ha^{-1} of P as super phosphate (46% P), the soil was ploughed to 30 cm depth soon after the first fall of rain with a disc plough, thereafter shallow tilled with a disk harrow, then with a tine harrow equipped with a roller cage. Wheat was sown after fallow in December using an experimental planter at a density of 350 seeds m^{-2} (Table 3). Total nitrogen fertilization was 100 kg N ha^{-1} as urea (46% N) split between three-leaf stage (40 kg N ha^{-1}) and early heading (60 kg N ha^{-1}). Depending on the years, the crop was harvested from late May to early June at grain moisture of 12 to 14% (Table 3). Every year, all the varieties were sown and harvested at the same time.

Irrigation

The optimal management of irrigation was determined previously, during seasons with roughly average precipitation. It became a guideline for the application of irrigation from 2007 to 2009, but with an adjustment for the earliness and intensity of annual rainfall deficit. Water was supplied by sprinklers across each plot.

The average rainfall deficit over the period from January to May during which irrigation takes place was 195 mm (105 to 300 mm depending on the year) (Table 1). The contribution of soil water due to the previous fallow was very variable from year to year. Moreover, the depth of the rooting zone did not exceed 60 cm in general due to the initial soil dryness and/or the low rainfall in autumn.

From local studies carried out in 2001 and 2003 where the yield responses of cv. Chen's and cv. Waha to increasing irrigation amounts were studied, the irrigation target for durum wheat was fixed at 180 mm as a compromise between the different cultivar responses. The intention was to meet the crop water needs during critical phases without consideration of varietal differences. Actual irrigation amounts and schedules were reported in Table 3.

Estimation of water use from a simple water balance model

As soil water content was not measured with probes or gravimetrically, a simple spreadsheet water balance model was built to calculate actual evapotranspiration (ETc) or consumptive water use for each year x water regime combination. Possible varietal differences were ignored. The water required to fully satisfy the crop requirement (ETmax) was calculated as usual as the product of ETo and Kc. The phasic crop coefficients (Kc) came from the FAO review of Doorenbos and Kassam (1979) but specific Kc values were not available to distinguish the six wheat genotypes (Bouthiba et al., 2008). We assumed that the crop reduced its transpiration linearly from ETmax once soil water content (SWC) fell below 2/3 ASW (that is, 121 mm), and ceased when ASW was exhausted (at a soil-water matric potential of -1.5 MPa) which is the norm in agro-climatic models (Brisson et al., 1992; Allen et al., 1998) as well as in irrigation scheduling methods (Bouthiba et al., 2008). Assuming a wheat root elongation of 1.2 mm per day after sowing (Paillard et al., 1992), the size of the reservoir accessible to roots increases steadily until heading. Assuming ASW to be zero at the end of summer in this region, SWC at wheat sowing was initialized by taking into account the P-ETo difference in October and November as this period has a direct influence on the water recharge of the profile.

Plant measurements

All the plant samplings and the organ counting were done at harvest on a microplot of 1.2 m² (6 rows) in the middle of the 6 m² sub-plot. The number of plants was determined after emergence and the number of ears (NE) at harvest. The number of grains per ear (NGE) was counted on a random sample of 15 ears for each sub-plot rather than by calculation from yield and individual grain weight. Grain yield (GY) was determined after mechanical threshing of wheat ears at plot harvest. From this grain bulk, the thousand grain weight (TGW) was determined. Grain number.m^{-2} was derived from NE and NGE and not from GY and TGW. Straw dry yield (SY) was determined after weighing the fresh non-grain fraction (leaves, stems and empty ears) and correcting by the moisture content of vegetative parts. Harvest index (HI) was calculated as the ratio of grain yield to total above-ground biomass at harvest.

Indicators of water productivity

We used here the terminology proposed by Oweis and Hachum (2006) to compare the efficiency of water for grain production between years, irrigation schemes and varieties. Thus, we speak of RWPg (Rain Water Productivity, kg of grain per m^3) for water efficiency resulting only from rainfall, of TWPg (Total Productivity Water, kg.m^{-3}) for water efficiency based on rain plus irrigation, and IWPg (Irrigation Water Productivity, kg.m^{-3}) for the marginal yield

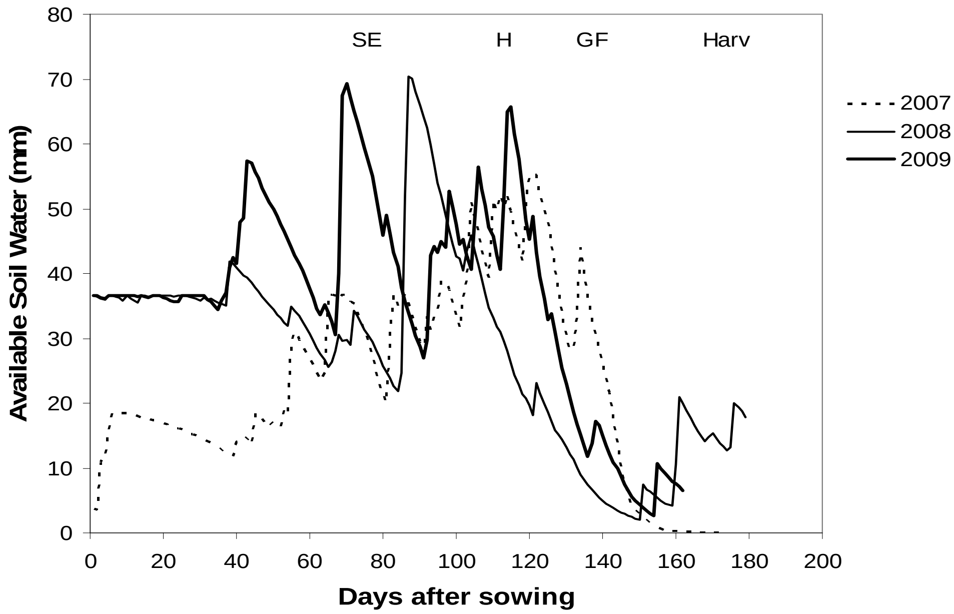

Figure 1. Time-course of the available soil water content (mm) during the 3 years (2007 to 2009) using a simplified soil water balance model. SE: onset of stem elongation; H: heading; GF: early grain filling; Harv: harvest.

gain allowed by irrigation alone.

Water use efficiency is generally calculated with an evapotranspiration term, either measured or simulated. As ETc did not result from soil water content measurements but from simulations, and because evapotranspiration was not estimated at variety level, we decided to use the previous ratios for comparing the performance of the wheat genotypes.

So, RWP was taken as the ratio of rainfed yield (grain or biomass) to rainwater, TWP was taken as the ratio of irrigated yield (grain or biomass) to total water supply (rain + irrigation), IWP was taken as the ratio of increase in yield (grain or biomass) to the amount of irrigation water applied.

Using the same assumptions, we calculated similar efficiency ratios for the production of straw: RWPs, TWPs and IWPs respectively.

Statistical analysis

Analyses of variance, stepwise and simple linear regressions and correlation analysis were all performed using the software Statistix 9.0 (Analytical Software, Tallahassee, FL, USA), and means were compared using the Least Significance Difference (LSD) method at $P < 0.05$. A split-split-plot design with three replications was used to analyze the combined effects of year, water regime and variety and their corresponding interactions. The main plot was 'year', the subplot was 'water regime' and the sub-subplot was 'variety'. This design confers to the 'variety' factor the highest degree of precision.

RESULTS

Characterization of water stress patterns

The intensity and timing of water deficit was analyzed using weather data (precipitation, evapotranspiration), simulated soil water content (obtained from a water balance sheet model) and yield component patterns in non-irrigated conditions.

Climatic and soil water deficit

During the two seasons 2006-2007 and 2007-2008 (October to June), the wheat crop received about the same amounts of rainfall (352 and 354 mm, respectively) as the 18-year average for that period (359 mm) (Table 1). However, the 2008 to 2009 season received 43% more rainfall than the average (513 mm).

To assess the year-to-year variation in water deficit, the dynamics of soil water content were simulated under non-irrigated conditions for the 3 growing seasons under study (Figure 1). A significant drought occurred in autumn 2006, characterized by a late start to the rains in 2007, which fell mainly from February to April (253 mm, Table 1).

The 2007-2008 season was characterized by high rainfall in autumn, a moderate water shortage in winter and spring and a rapid soil water depletion before heading. In contrast, the 2008-2009 season was characterized by uniform but moderate water availability throughout the growing season. During the period of establishment of the number of grains per ear, the 2009 conditions were obviously the most favorable for yield. In May and June, rainfall was extremely deficient whatever the season (< 35 mm) but the largest water deficit was observed in 2008.

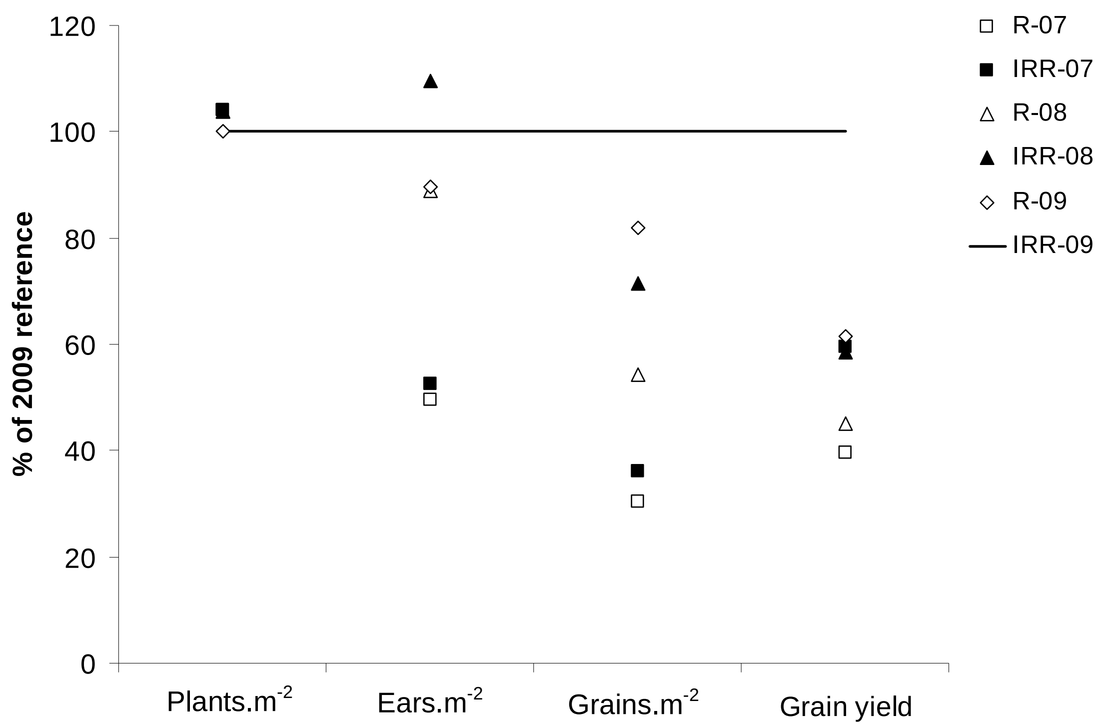

Figure 2. Annual pattern of yield build-up under rainfed (R) and irrigated (IRR) wheat management; the yield component values are expressed as relative values of 2009 irrigated reference (average data on 6 durum wheat cultivars) – 07, 08 and 09 refer to 2007, 2008 and 2009 growing seasons.

Yield components' patterns

According to the seasonal rainfall, the year-to-year variability of wheat yield was pronounced in the semi-arid conditions of Upper Chelif, resulting in rainfed grain yields ranging from 1.8 t.ha^{-1} (2007) to 2.8 t.ha^{-1} (2009) for the mean of all varieties, and from 0.7 to 3.4 t.ha^{-1} when considering all the variety x season combinations.

It is generally agreed that wheat yield results from four successive steps: (i) the establishment of the plant population (NP), (ii) the establishment of the number of ears per m² (NE) achieved at heading, (iii) the establishment of the number of grains per m² (NG) completed in early grain filling, (iv) grain filling, established at physiological maturity (GY). To identify the phenological phases that were affected by water shortage, we broke down the elaboration of wheat yield in the form of a chronological pattern, expressing each yield component (plants.m^{-2}, ears.m^{-2}, grains.m^{-2}, grain yield) relative to its reference value measured under fully irrigated conditions. The yield build-up pattern with time is shown in Figure 2 for unirrigated wheat by averaging the data of the six wheat genotypes. The component reference was given by the yield components under irrigated conditions in 2009, the season considered to express the yield potential.

In 2007, drought was severe during shooting, which resulted in a small number of ears.m^{-2} (49% of the reference). In 2008 and 2009, water stress was less pronounced and occurred later during the formation of the number of grains per ear (NE at 89-90% of the reference, but NG at 71 and 82% of the reference in 2008 and 2009, respectively). Thus, under a rainfed regime, yield establishment gradually diverged from the optimum, with water stress increasing during the season. The final GY was 40 to 61% of the reference value (IRR-09).

Response of durum wheat yield to water

In irrigated conditions, the maximum yield (4.5 t.ha^{-1}) was observed in 2009, associated with maximal water use (380 mm). In rainfed conditions, maximum yield was 2.8 t.ha^{-1} in 2009 and water use did not exceed 250 mm. Corresponding values for above-ground biomass (grain + straw) at harvest were 11.4 t.ha^{-1} (irrigated) and 8.1 ha^{-1} (rainfed).

The average annual yield (GY, t.ha^{-1}) of durum wheat, all varieties averaged, increased linearly with actual evapotranspiration (ETa, mm) whatever the origin of water, either rainfall or irrigation (Figure 3a). The relationship obtained was: GY = 0.0118 ETa - 0.21 (r^2 = 0.89, n = 6, $P < 0.01$). From Figure 3a, a mean crop water productivity value (GY/ETa) of 1.10 kg.m^{-3} was determined for durum wheat.

The relationship was also linear when total above-ground biomass (or total dry matter, TDM) was used as

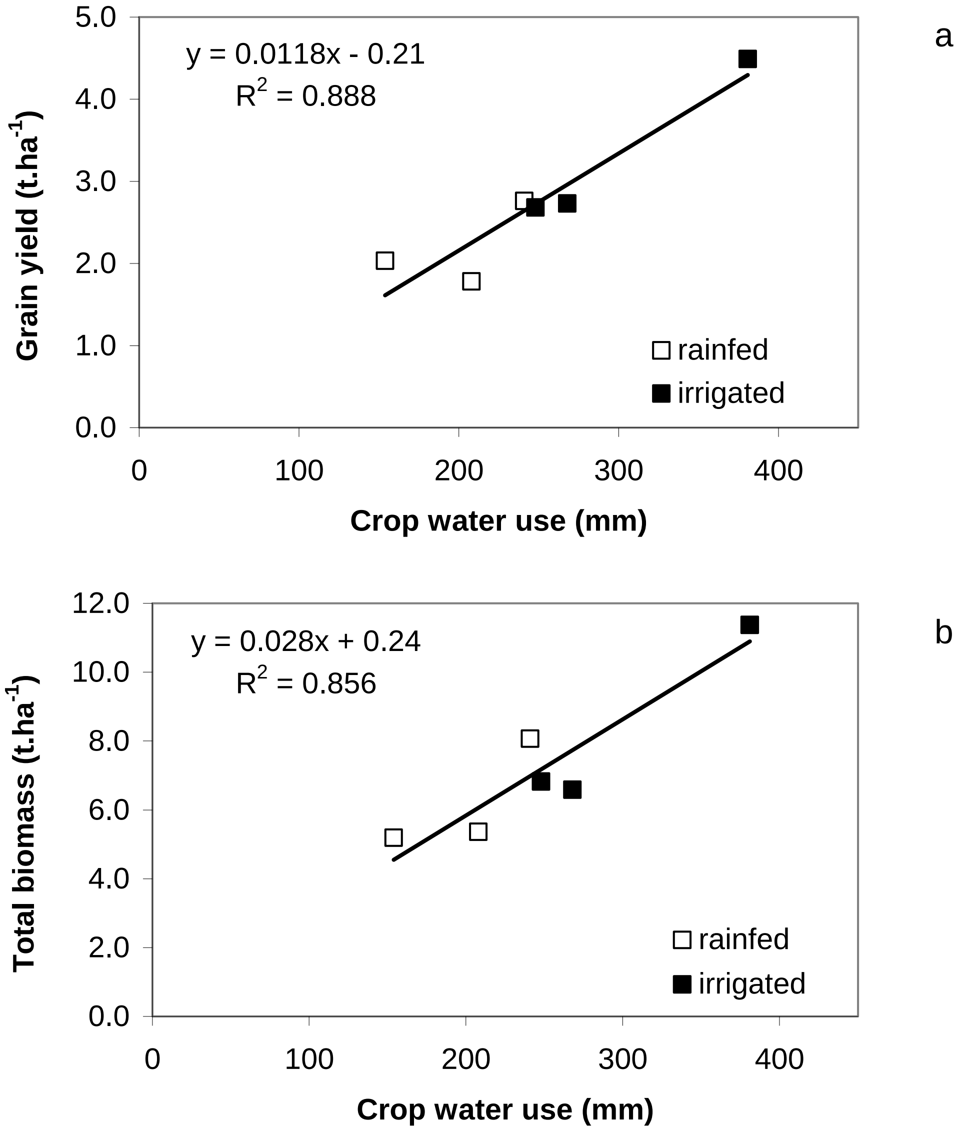

Figure 3. Relationship between average (a) grain yield ($t.ha^{-1}$) or (b) total above-ground biomass ($t.ha^{-1}$) and crop water use (mm) calculated by a simple water balance model; the 6 varieties under test are averaged; each point corresponds to a year x water regime combination.

an indicator of crop growth (Figure 3b). The relationship obtained was: TDM = 0.028 ETa + 0.24 ($r^2 = 0.86$, n = 6, $P < 0.01$). From Figure 3b, the mean crop water productivity value (TDM/ETa) was 2.89 $kg.m^{-3}$.

Wheat response to irrigation according to year and strategy

Grain yield

Grain yield differed significantly ($P < 0.001$) between years, water regimes and varieties. Significant interactions were found between irrigation response and year ($P = 0.014$), and between variety and year ($P < 0.001$). The irrigation and variety main effects were both significant at $P < 0.05$ for the three years under test, but no irrigation x variety interaction was found.

Irrigation gave a yield increase of 0.7 to 1.7 $t.ha^{-1}$ according to year and scheduling strategy (Table 4). The extra yield obtained by irrigation (50 mm in 2007, 180 mm in 2008 and 2009) was generally less than 1.0 $t.ha^{-1}$ except in 2009 when 1.5 to 2.5 $t.ha^{-1}$ was obtained, depending on genotype.

The interaction between water regime (irrigation) and variety for yield was not statistically significant in 2007,

Table 4. Response of grain yield ($kg.ha^{-1}$) to variety for the three growing seasons 2007, 2008, and 2009 and the two water regimes (rainfed, irrigated).

Water regimes	Bousselem	Chen's	Mexicali	Mohammed Ben Bachir	Vitron	Waha
Rainfed						
2007	3017^{a}	2283^{b}	1200^{c}	1633^{c}	1317^{c}	1217^{c}
2008	1904^{b}	2670^{a}	2234^{ab}	690^{c}	2144^{b}	2140^{b}
2009	3400^{a}	2500^{bc}	3333^{a}	1733^{c}	2500^{bc}	3100^{ab}
3 years	2774^{a}	2485^{ab}	2256^{ab}	1352^{c}	2113^{b}	2152^{b}
Irrigated						
2007	3460^{a}	3132^{ab}	2033^{c}	2532^{abc}	2150^{bc}	2951^{abc}
2008	2268^{b}	3425^{a}	3142^{a}	1210^{c}	3085^{a}	3052^{a}
2009	5133^{a}	4933^{ab}	4767^{ab}	2800^{c}	4033^{b}	5300^{a}
3 years	3620^{ab}	3767^{a}	3314^{ab}	2181^{c}	2963^{b}	3767^{a}

Data followed by the same letters in lines are not significant at P < 0.05.

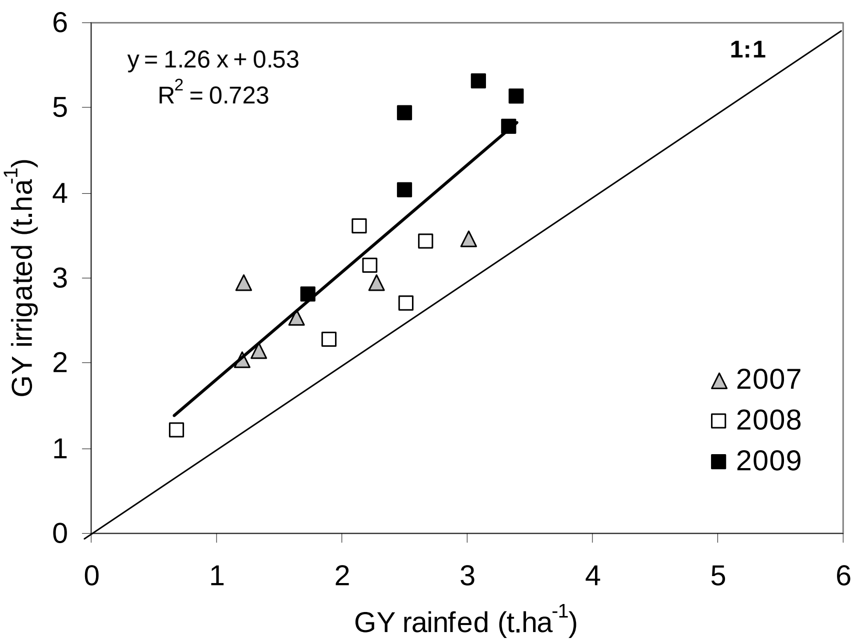

Figure 4. Relationship between irrigated and rainfed grain yield (GY, $t.ha^{-1}$) for a range of 6 durum wheat varieties and 3 growing seasons (2007, 2008, 2009).

2008 and 2009, when it was tested. No irrigation x variety interaction was found at $P < 0.05$ when pooling all the seasons in the analysis of variance. However, the variety x year interaction was significant, which suggests that genotype x environment interactions may be observed as a result of differential water deficit patterns and genotype characteristics. Due to the absence of a variety x irrigation interaction, yields of irrigated and rainfed wheat were strongly correlated when comparing the effect of water regime for the 6 varieties and 3 growing seasons ($r^2 = 0.73$, n = 18, $P < 0.001$) (Figure 4).

Yield components' responses to irrigation were analyzed after averaging all the varieties under test as no variety x irrigation interaction was observed for each of the yield components (Table 5). In 2007, the contribution of an early irrigation of 50 mm significantly increased the number of grains per ear (+13%) with positive effects on yield. However, the number of ears per m^2 was slightly increased on average, but leveled out at less than 200. In 2008, the positive effects of irrigation were apparent on the number of ears per m^2 (+25%). In 2009, irrigation had a positive but limited effect on the number of ears per m^2 (+11%) and the number of grains per ear (+9%). Due to the increased number of grains per m^2 with irrigation in

Table 5. Response of yield components to irrigation (the 6 varieties were pooled as no variety x irrigation interactions were observed for the yield components).

Yield components	2007		2008		2009	
	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated
Ears m^{-2}	184	195	325^{b}	408^{a}	334^{b}	372^{a}
Grains ear^{-1}	33.6^{b}	37.8^{a}	33.1	35.5	49.6^{b}	54.3^{a}
Grains.m^{-2}	6177^{b}	7259^{a}	10724^{b}	14494^{a}	16561^{b}	20228^{a}
Thousand grain weight (g)	33.9	37.1	34.5	34.7	32.9	35.9

Within a year, the data followed by the same letters in lines are not significant at P < 0.05.

Table 6. Indicators of crop water productivity for grain (kg.m^{-3}) as a function of variety and year : 2007, 2008, 2009.

Year	Bousselem	Chen's	Mexicali	Mohammed Ben Bachir	Vitron	Waha	Total
RWPg							
2007	0.96	0.72	0.38	0.52	0.42	1.09	0.68
2008	1.03	1.44	1.21	0.37	1.16	1.16	1.06
2009	1.17	0.86	1.14	0.59	0.86	1.06	0.95
3 years	1.05	1.01	0.91	0.49	0.81	1.10	0.90
TWPg							
2007	0.95	0.81	0.56	0.69	0.59	1.05	0.78
2008	0.62	0.94	0.86	0.33	0.84	0.84	0.74
2009	1.09	1.05	1.01	0.59	0.86	1.12	0.95
3 years	0.89	0.93	0.81	0.54	0.76	1.00	0.82
IWPg							
2007	0.89	1.32	1.67	1.80	1.63	0.81	1.35
2008	0.20	0.42	0.50	0.29	0.52	0.51	0.41
2009	0.96	1.35	0.80	0.59	0.85	1.22	0.96
3 years	0.68	1.03	0.99	0.89	1.00	0.85	0.91

RWP = Rain water productivity; TWP = total (rain + irrigation) water productivity; IWP = irrigation water productivity.

2007 (+17%), 2008 (+35%) and 2009 (+22%), the individual grain weight increased very little with irrigation, the differences in this component being non significant with rainfed management.

Thus, under irrigation, the yield formation patterns were relatively similar throughout the cycle, mainly because of irrigation schedules starting at heading (Figure 2). Grain number.m^{-2} was the yield component systematically improved by deficit irrigation applied between shooting and booting (Table 5).

In 2007, because of early drought, the efficacy of irrigation at tillering (50 mm) was very high: IWPg increased to 1.35 kg.m^{-3} (Table 6). The same amount of water (180 mm) in 2008 and 2009 did not result in the same value of IWPg for the 2 contrasting seasons: 0.34 vs. 0.96 kg.m^{-3}.

Straw yield and harvest index

Irrigation significantly increased straw yield (SY, P < 0.001) and harvest index (HI, P < 0.05). As in the case of grain yield, a year effect was clear for these two variables. SY and HI differences with irrigation were significant at P < 0.05 in 2007 and 2009 but not in 2008.

Irrigating between shooting and booting resulted in an increase in straw yield of between 16% (2007) and 30% (2009), and averaged over the 6 varieties. In 2007 and 2008, straw yield was about 4 t.ha^{-1} while up to 7 t.ha^{-1} was observed with irrigation in 2009. At the same time, harvest index (HI), which ranged from 0.34 to 0.38 under rainfed management, attained 0.40 to 0.42 with irrigation.

Similar to grain yield, no significant interaction between irrigation and variety was observed in the three experiments, but the interaction was significant between variety and year for straw yield and harvest index (P < 0.001).

Varietal response to available water

The performance of the six durum wheat varieties was

Table 7. Response of straw yield (kg.ha^{-1}) to variety for the two water regimes (rainfed, irrigated) – Average values over the 3 growing seasons.

Water regimes	Bousselem	Chen's	Mexicali	Mohammed Ben Bachir	Vitron	Waha	Average
Rainfed	3842^{b}	3755^{b}	3458^{b}	5724^{a}	3655^{b}	3656^{b}	4015
Irrigated	5173^{b}	4323^{b}	4583^{b}	6867^{a}	4227^{b}	4574^{b}	4958

Data followed by the same letters in lines are not significant at $P < 0.05$.

compared for 3 years (2007 to 2009) under rainfed and irrigated conditions.

Varietal response in rainfed management

Under rainfed management, the variety Mohammed Ben Bachir (MBB) was the least productive in 2008 and 2009 (Table 4) but with early drought (2007) MBB was less adversely affected than the other varieties, although its yield did not exceed 2.0 t.ha^{-1}. The Chen's variety had very stable yields, always above 2.0 t.ha^{-1}. Cv. Bousselem achieved its high potential yield (> 3.0 t.ha^{-1}) in 2007 and 2009. Cvs Mexicali, Waha and Vitron were characterized by higher yield variability under rainfed management, with yields similar to Chen's and Bousselem only in 2009, when water stress was moderately limiting.

Water productivity in rainfed conditions (Table 6) was higher for Waha, Bousselem and Chen's (RWP between 1 and 1.1 kg.m^{-3}) than for Mexicali and Vitron (0.8 to 0.9 kg.m^{-3}) and the local variety Mohammed Ben Bachir (0.5 kg.m^{-3}). Using the indicator TWPg, genotypes ranked the same but the values were lower except for the local variety.

In 2007, when drought occurred early, RWPg was low on average (0.68 kg.m^{-3}). However, the two varieties Bousselem and Waha had an efficient use of rainwater in this situation (RWPg = 0.96 and 1.09 kg.m^{-3}, respectively). In 2008, a late drought year, the best value was obtained for cv. Chen's under rainfed (RWPg = 1.44 kg.m^{-3}), while the late-maturing variety MBB was strongly penalized (0.37 kg.m^{-3}). In 2009, a year with well-distributed rainfall, RWPg was lower than in 2008, and the varieties Chen's, Vitron and MBB were the least efficient for rainfall use.

Varietal response to irrigation

Although varietal differences were rather small, we can nevertheless separate three types of response to wheat irrigation on the basis of water productivity indicators for grain (Table 6).

Cvs Bousselem and Waha, as early drought-tolerant varieties ($RWPg_{2007}$ = 0.96 and 1.09 kg.m^{-3}), did not respond well to early irrigation ($IWPg_{2007}$ = 0.89 and 0.81 kg.m^{-3} respectively): this type of variety requires later irrigation, which is efficiently converted into yield.

Cv. Chen's variety is characterized by an average tolerance throughout its life, with a stable response to water, whether from rain or irrigation (RWPg = 1.01 kg.m^{-3}, TWPg = 0.93 kg.m^{-3}, IWPg = 1.03 kg.m^{-3}). This variety responds well to a moderate water supply distributed evenly throughout growth. It responds best to regular irrigation regardless of drought patterns.

Cvs. Mexicali and Vitron are very sensitive to early drought ($RWPg_{2007}$ = 0.38 and 0.42 kg.m^{-3}, respectively), and require large water inputs as early as tillering if a drought begins then. The efficacy of such early irrigation is particularly high ($IWPg_{2007}$ = 1.67 and 1.63 kg.m^{-3} respectively).

MBB, a late maturing variety with a low yield potential, responded poorly to irrigation except in the case of an early drought. But the good response in 2007 ($IWPg_{2007}$ = 1.80 kg.m^{-3}) did not result in a sufficient yield increase because of its low yield potential (Table 4). Indeed, its potential remained below 3.0 t.ha^{-1} when the season was fully conducive to crop growth as in 2009.

The best overall response to available water (TWPg) was obtained for the variety Waha (1.00 kg.m^{-3}) followed by Chen's (0.93 kg.m^{-3}) and Bousselem (0.89 kg.m^{-3}).

Straw production as affected by variety

The local variety MBB produced 50% (irrigated) and 56% (rainfed) more straw biomass than the 5 other introduced cultivars (Table 7). Among the latter, no significant difference in straw yield was observed, although more straw was obtained with irrigation: from 3.4 to 3.8 t.ha^{-1} in non-irrigated treatments, from 4.2 to 5.2 t.ha^{-1} with irrigation. This was in accordance with the plant height, which averaged 90 cm for MBB and ranged from 73 cm (Chen's) to 82 cm (Mexicali) for the other cultivars.

Consequently the local variety MBB was characterized by a very low harvest index: 0.19 vs 0.36 to 0.43 (5 varieties) in unirrigated plots, 0.25 vs 0.41 to 0.47 (5 varieties) in irrigated plots. Among the 5 varieties, no significant variety effect was observed for HI and there was no variety x irrigation interaction.

The best overall response to available water for straw production (TWPs) was clearly observed for MBB (2.15 kg.m^{-3}), while the introduced varieties had much lower

Table 8. Significant coefficients at P < 0.05 of the stepwise linear regressions between grain yield and its main components.

Water regimes	R²	Constant	Plants.m^{-2}	Ears.m^{-2}	Grains.ear^{-1}	Thousand grain weight
Total	0.652	-17.21*	-0.057*	0.049***	0.448***	0.809***
Irrigated plots	0.703	-35.30***		0.036*	0.625***	0.871***
Rainfed plots	0.598	-2.19 ^{ns}		0.045*	0.301*	

ns: not significant ; * (P < 0.05) ; ** (P < 0.01) ; *** (P< 0.001). The missing cases correspond to variables not included in the model at P < 0.05.

values, ranging from 1.36 to 1.49 kg.m^{-3}. The cultivar Mexicali had a balanced response to irrigation in terms of straw and grain as IWP was 0.83 kg.m^{-3} for straw and 0.99 kg.m^{-3} for grain while MBB was efficient for straw production and Chen's and Vitron were efficient for grain production.

Statistical model of grain yield formation

A stepwise linear regression was performed between grain yield and its main components (at P <0.05) (Table 8). It is thus clear that under rainfed management, the yield components which are determined first, such as the number of ears per m² (NE) and the number of grains per ear (NGE), are the most explanatory of final grain yield. With irrigation, the components NGE and thousand grain weight (TGW), which is determined last, are more explanatory of the final yield. Moreover, the correlation coefficient of yield with plant density was higher in rainfed than in irrigated conditions, because partial compensation is possible by other components if water is available. Under a rainfed regime, the change in TGW had less impact on yield than under irrigation, because most of the yield variation was attributed to grains.m^{-2}.

DISCUSSION

Overall response of durum wheat to water use

The different weather scenarios and irrigation strategies were combined to examine the relationship between yield and water use for a wide range of yield and ETa values. The value of crop water productivity (GY/ETa) of 1.1 kg.m^{-3} that was derived from Figure 3a was in accordance with the average value given for wheat by Zwart and Bastianssen (2004). Their comprehensive review concealed however a wide variation in this indicator of water use efficiency. In previous literature reviews, Doorenbos and Kassam (1979) and Musick and Porter (1990) indicated common values between 0.8 and 1.2 kg.m^{-3}. In Algeria and Tunisia respectively, Bouthiba et al. (2008) and Rezgui et al. (2005) mentioned values of 0.5 to 1.4 kg.m^{-3} depending on climatic zones, irrigation schedules and varieties. The good correlation between GY and ETa and the agreement of the crop water productivity ratio with previous reports suggest that the simple water balance model we used to calculate crop evapotranspiration was reasonably realistic in these soil conditions.

Differential sensitivity of wheat to drought according to the physiological phases

The very contrasting drought scenarios under rainfed management made possible the appraisal of water deficit effects on the wheat crop. The early drought in 2007, which seriously reduced the number of plants and the number of ears per m², greatly reduced the final yield. According to El Hafid et al. (1998b), water stress at tillering stops tiller emission and reduces the growth of tillers already formed. In addition, severe water stress reduces the length and volume of seminal roots, mainly in the deeper layers of soil, reducing the available water for wheat during the second part of the growing season (Adda et al., 2005). All this leads to increased sensitivity of the wheat plants during later periods of water shortage and in reductions in the potential yield which are difficult to compensate for later by irrigation.

The most common drought scenario was described as drought increasing during shooting with a consequent reduction in ear fertility and/or individual grain weight, depending on the earliness and intensity of water shortage. The regression of tillers during shooting can also affect greatly the number of ears (Debaeke et al., 1996). The representation of the yield formation pattern in Figure 2 illustrates clearly the gradual divergence of the yield component pattern from the potential production target defined in conditions with a regular precipitation distribution. We feel that this simple representation facilitates the comparison and analysis of the effect of water deficit between seasons, management options and varieties.

The varieties tested were differently affected by the drought scenarios according to their morphological, physiological and phenological traits and their potential pattern of yield formation. Among the tested varieties, previous studies have already discussed the importance of certain traits for conferring drought tolerance to durum wheat cultivars. In a 4-year study, Bouthiba et al. (2008) recommended cvs. Chen's and Waha in conditions of moderate stress, while cv Vitron performed better under full irrigation. Comparing two cultivars under various water deficit treatments, Larbi et al. (2000) concluded that Waha was relatively drought-tolerant during shooting compared to Vitron. David (2009) estimated the osmotic adjustment capacity of several durum wheat cultivars grown in Algeria, based on pollen grain expression, and concluded that MBB, Bousselem and Chen's had high drought tolerance, Waha and Mexicali intermediate tolerance, and Vitron low tolerance. Among the varieties tested in this study, Mexicali and Vitron were inefficient in converting available water into grain (low RWPg) and Waha and Mexicali had low values of RWP for straw which is in agreement with the study of David (2009).

Response of wheat to irrigation

Irrigation increased the average rainfed yield of durum wheat by 1.1 $t.ha^{-1}$ (0.4 to 2.4 $t.ha^{-1}$ depending on the year, mean of all varieties). The mean yield achieved with irrigation was 3.3 $t.ha^{-1}$ compared with 2.2 $t.ha^{-1}$ for rainfed crops, an increase of 60%, using 140 mm irrigation on average.

Studies in the WANA region on durum wheat sometimes gave larger increases. Thus, in Algeria, Bouthiba et al. (2008) obtained a yield increase of 270% (rainfed yield : 1.3 $t.ha^{-1}$) with full irrigation (270 mm), 107% for irrigation prior to heading (130 mm) and 67% for post-heading irrigation (140 mm). In Syria, Oweis et al. (1999) observed increases of 45, 71 and 80% of rainfed yield (2.6 $t.ha^{-1}$) for irrigation programs covering 1/3, 2/3 and full water requirements of durum wheat (320 mm irrigation).

However, the irrigation program in this study covered only part of the water requirement of wheat, corresponding more to deficit irrigation than to supplementary irrigation *sensu stricto* (Geerts and Raes, 2009). By limiting water applications to drought-sensitive growth stages, this practice aims to maximize water productivity and to stabilize - rather than maximize – wheat yields (Khila et al., 2013). In Algeria, cereals are rarely grown under full irrigation, the common practice being to use small amounts at critical stages to prevent crop failure. Zhang and Oweis (1999) showed that the periods of maximum sensitivity of durum wheat to drought are between the onset of stem elongation to booting, and then from anthesis to dough grain, hence the importance of ensuring good water supply during these periods.

In 2007, the addition of 50 mm at tillering helped increase the yield by 52% in a situation where drought compromised wheat yield as early as the establishment phase. In Turkey, Ilbeyi et al. (2006) also obtained a 65% yield increase from early irrigation. This was related to the combined action of water deficit on early growth of roots and shoots, as well as the initiation of leaves and reproductive organs, the potential size or number of which might be limited.

The different types of drought (early, late) characterizing the semi-arid Algerian area appear randomly and with varying intensities. The choice of varietal earliness at heading should result from a frequency analysis, and the use of simulation models might be of great help (e.g. Rezzoug et al., 2008). Among the varieties tested, Mohammed Ben Bachir matured the latest: although it may tend to escape early stress, it is likely to suffer towards maturity by the action of high temperatures that shorten the duration of filling. Thus, post-flowering photosynthesis can be severely reduced; therefore, delayed irrigation should benefit this kind of variety. A possible but partial compensation of grain growth could be achieved by the remobilization of sugars from the stem. Latiri et al. (2013) pointed out the buffering effect of this process for maintening grain yield in water-limited conditions.

In rainfed conditions, the yield components determined early in the season - the number of ears (NE) and number of grains per ear (NGE) - are more explanatory of grain yield because they are more strongly affected by water deficit (Table 8). The individual grain weight is less depressed as it benefits from a reduction of the number of sinks, resulting in a high value of the source : sink ratio for carbon.

In irrigated conditions, the three yield components (NE, NGE, TGW) are all involved in the formation of yield. These observations confirm those of Garcia del Moral et al. (2005) from a 'path analysis' applied to the performance of 25 genotypes in irrigated and dry conditions. These authors showed that in irrigated conditions, grain yield depends equally on the three components NE, NGE and TGW, while in dry conditions, the variation in performance is mainly due to NE and to a lesser extent to NGE. In dry conditions, the production of tillers limits NE while in irrigated conditions the regression rate of tillers is the cause of lower NE. Overall, it was confirmed that irrigation increases all the yield components, with a consistent effect on NGE.

However, wheat irrigation could have some limitations in the conditions of semi-arid Algeria. Indeed, these areas are often subject to climatic hazards such as very dry air, hot winds and high temperatures that generate massive spikelet abortion and high rates of shriveling, exceeding 50% of the harvest. In our multi-year study, one year in four was subject to these problems. In these situations, the impact of irrigation could be negligible among the least drought-tolerant varieties.

Conclusion

From this 3-year study, it can be concluded that the varieties Mexicali and Vitron, which were very unreliable in rainfed conditions, should always be irrigated, while only varieties with a good yield potential and a fair tolerance to drought, such as Bousselem and Chen's, can make full use of deficit irrigation, especially if it is applied between the end of shooting and the soft dough grain stage.

The development of deficit irrigation on durum wheat when adapted to variety choice and related crop management aspects could help to reduce the gap between actual and attainable grain yield and the minimization of the year-to-year variability in wheat yield in Algeria. Under experimental conditions over three years, a mean grain yield of 3.3 $t.ha^{-1}$ could be attained with irrigation in the Upper Chelif region (instead of 2.2 $t.ha^{-1}$ under rainfed conditions) with an irrigation efficacy of 0.79 $kg.m^{-3}$.

However, the scarcity of water resources in north Africa and their priority for domestic uses and horticultural cash crops could limit the access to irrigation and climatic stresses could reduce its profitability on cereals. Therefore, Oweis et al. (1999) recommended irrigation to between one third and two thirds of total requirement of wheat, for reasons of efficiency and profitability. In addition, plant breeding should be focused to develop wheat genotypes with stable behavior under moderate drought but a good response to deficit irrigation when necessary.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Chromosomal characteristics of Tho-Tho cattle (Bos indicus) in Nagaland, India

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Tho-Tho cattle is a semi-wild indigenous cattle available in Nagaland, India. Tho-Tho cattle has been associated with tribal culture of 16 tribes of Nagaland besides its use for meat, milk and draught purposes. A cytogenetic analysis was conducted in a total of 10 numbers of Tho-Tho cattle (5 males and 5 females) from 3 districts of Nagaland hills situated at mean sea level (msl) ranging from 1000 to 10000 ft (4 nos. from one district and 3 nos. each from other 2 districts). The statistical design included 5 males and 5 females Tho-Tho cattle for gathering data on cytogenetic parameters viz. (i) karyotype, (ii) relative length, centromeric index and arm ratio of chromosome and (iii) construction of ideograms. Statistical tools used for analysis of variation of relative length of each autosome, centromeric index; arm ratio of the 'X' chromosome between male and female are done by using the Student's t-test. Duncan's multiple range test (DMRT) was used to isolate means if the effect was found significant. The study revealed that- (i). Tho-Tho cattle has 29 pairs of autosomes, (ii) out of 29 pairs, 23 pairs of autosomes did not differ significantly ($P > 0.05$) between male and female Tho-Tho cattle in terms of relative length., (iii) autosomes are acrocentric, (iv) relative length of 4th, 5th, 6th, 7th and 9th autosomes are significantly ($P < 0.05$) higher in male; however, 19th autosome of female is significantly ($P < 0.05$) higher than male, (v) X-chromosome in both the sexes is clearly distinguishable and is submetacentric, (vi) Y-chromosome (to the total haploid genome) of male Tho-Tho cattle is 1.77 ± 0.10% and it is acrocentric (vii) centromeric index of "X" chromosome in male is 33.75% and in female 32.76% and (viii) Arm ratio of "X" chromosome is found to be 0.66 in male and 0.67 in female.

Key words: Centromeric index, Y-chromosome, X-chromosome, sexes, Tho-Tho cattle.

INTRODUCTION

Tho-Tho cattle is a local name of a semi wild indigenous cattle, which is found in Sub Himalayan region of North Eastern India, particularly in Nagaland hills at an altitude of 1000 to 10000 ft. msl. No literature is available on Tho-Tho cattle as no research has been done yet on this animal. Tho-Tho cattle are popular by different names for different tribes within their own dialects. This cattle is medium in structure (average length in male is 108.2 ± 1.5 cm and in female 106.8 ± 1.7 cm and average height in male is 120.4 ± 1.6 cm and in female 109.8±1.5 cm) and closely associated with tribal rituals, religious and socio economic activities of Naga tribes. This animal is

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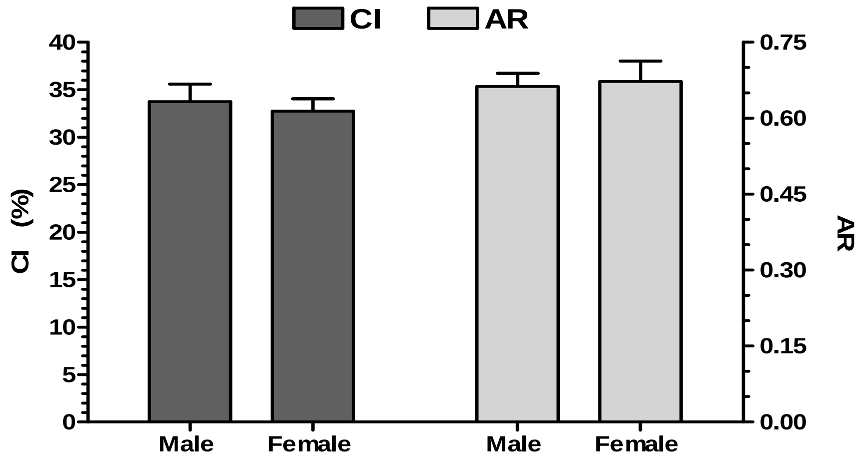

Figure 1. Variation (mean±SE) in centromeric index (CI, %) and arm ratio (AR) of X chromosome in male and female *Tho-Tho* cattle.

mainly used for meat purpose and also for dairy and tilling and draught purposes. Currently, meagre scientific information is available on the qualitative and quantitative traits of this animal. The animal did not have attract due attention from the appropriate authority due to its remote habitat and semi wild behaviour. Therefore, this study was designed with an objective of investigating chromosomal characteristics to explore its potential as domestic cattle. Investigation on chromosomal profile in livestock provides a useful tool to identify and judge the fertility status of the animal even at an early age (Basumatary, 2003). The knowledge of chromosomal characteristics of Tho-Tho cattle and their effects on economic traits will also be helpful in planning animal breeding strategies.

METHODOLOGY

A cytogenetic analysis was conducted in a total of 10 Tho-Tho cattle (5 males and 5 females) from 3 districts of Nagaland hills situated at mean sea level (msl) ranging from 1000 to 10000 ft (4 from one district and 3 each from other 2 districts). Based on the secondary data on habitat of Tho-Tho cattle collected from Department of Animal Husbandry and Veterinary, Forest and environment, National Research Centre on Mithun and village heads; Kohima, Phek and Zunheboto districts of Nagaland were selected for the survey. Sample size was purposefully fixed at 5 males and 5 females. Cytogenetic parameters studied were (i) karyotype, (ii) relative length, centromeric index and arm ratio and (iii) construction of ideogram. For karyotype study a total of 100 karyotypes (50 from 5 males 50 from 5 females) were prepared to study the morphometry of chromosomes following the protocol described by Moses (1974).

Karyotype preparation

The chromosomes were counted and numbered on the printed copy of the image. Length of each chromosome was determined using the image analysis software of the Nikon microscope (Eclipse 80i) and digital copy of the image. The homologous pairs of chromosomes were identified by matching their absolute length. The chromosome pairs were then arranged in a decreasing order of length and a digital image of the full set was prepared using the Adobe photoshop software.

Estimation of relative length, centromeric index and arm ratio of the chromosomes

Length of chromosomes was measured from centromere to telomere in case of acrocentric chromosomes and tip of short arm to the tip of long arm in case of submetacentric chromosomes. Length of the homologous chromosomes; X-chromosome in female and in case of males, lengths of "X" and "Y" were measured separately and calculated using the formula (Mean±SE) described by Choudhury et al. (1997). The centromeric index of the submetacentric chromosomes was calculated using the formula (Choudhury et al., 1997).

Construction of idiograms

A total of 30 karyotypes from 5 males and 5 females were taken for preparation of idiograms (Figures 2 and 3) as described by Rowley (1974).

Statistical tools used were for analysis of variation of relative length of each autosome, centromeric index; arm ratio of the 'X' chromosome between male and female are done by using the Student's t-test. Duncan's multiple range test (DMRT) was used to isolate means if the effect was found significant.

RESULTS

Ecological and environmental conditions are presented in Table 1. The results of the cytogenetic characters are presented in Table 2. The cytogenetic analysis of Tho-Tho cattle revealed a diploid number of 60 XX in female and 60 XY in male. The morphometric measurements obtained from each pair of chromosomes and sex chromosomes were expressed in terms of relative length

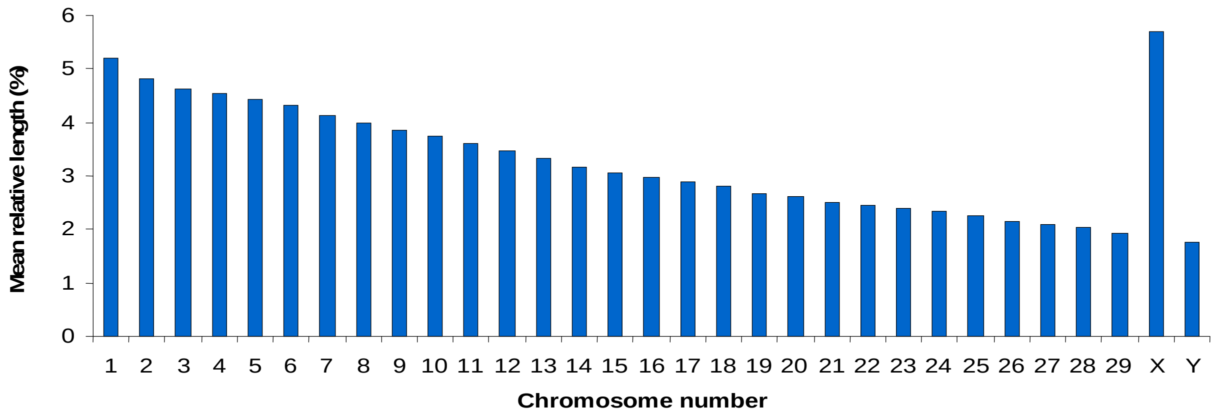

Figure 2. Idiogram of male Tho-Tho cattle.

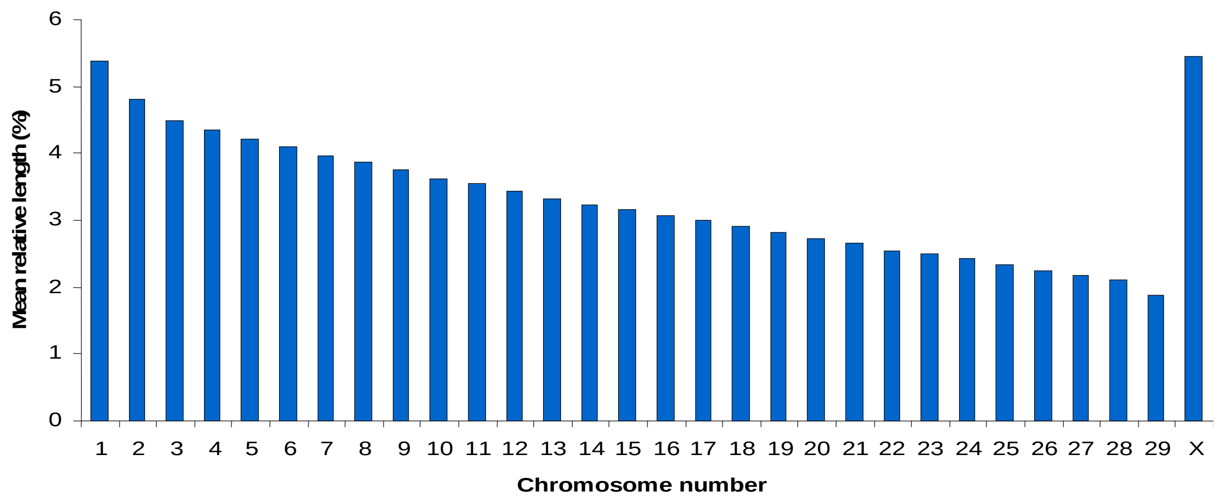

Figure 3. Idiogram of female Tho-Tho cattle.

Table 1. Month wise variation in average temperature, dew point, relative humidity and rainfall in different districts.

Particulars	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Kohima District												
Average temperature (°C)	10.2	12.2	16.1	19.3	20.6	21.6	22.4	22.1	21.2	18.9	15.4	11.7
Average Dew Point (°C)	6.8	7.7	12.4	12.8	14.9	16.7	18.5	19.8	19.7	18.3	15.7	12.5
Average relative humidity (%)	61.1	60.1	58.6	63.1	74.3	80.5	82.9	82.8	82.4	77.4	71.8	62.7
Average rainfall (mm)	15.2	10.5	34.5	162.1	148.2	238.3	377.4	290.4	232.4	70.4	22.3	20.7
Phek District												
Average temperature (°C)	11.5	13.9	17.1	17.6	20.1	21.8	22.2	22.7	21.7	19.1	15.5	12.2
Average Dew Point (°C)	5.6	8.4	9.6	12.8	13.7	16.8	18.3	19.1	18.2	16.4	13.6	10.5
Average relative humidity (%)	71.1	47.8	64.2	74.3	75.5	80.8	85.2	82.9	84.7	82.5	83.3	78.2
Average rainfall (mm)	16.6	10.1	30.1	178.3	190.5	193.9	344.5	296.1	180.6	172.1	26.6	21.7
Zunheboto District												
Average temperature (°C)	9.7	11.5	13.4	14.2	15.2	18.2	19.2	19.2	18.4	17.9	15.8	13.7
Average Dew Point (°C)	4.1	6.5	10.2	9.8	10.1	12.7	14.7	16.6	17.1	15.7	12.6	9.3
Average relative humidity (%)	88.2	85.1	88.2	85.8	87.7	89.9	92.9	95.3	90.8	89.9	84.8	58.3
Average rainfall (mm)	33.5	18	46	279.6	298.3	318.3	410.8	443.2	299.7	133.6	41.3	1.3

Table 2. Relative length (mean ±SE) of the chromosomes (haploid set) of *Tho-Tho* cattle.

Chromosome pair no.	Relative length (%)	
	Male (Mean ± SE)	Female (Mean ± SE)
1	5.19 ± 0.12	5.38 ± 0.16
2	4.83 ± 0.05	4.81 ± 0.08
3	4.63 ± 0.05	4.49 ± 0.03
4	4.53 ± 0.04[a]	4.34 ± 0.04[b]
5	4.42 ± 0.06[a]	4.22 ± 0.05[b]
6	4.31 ± 0.05[a]	4.09 ± 0.04[b]
7	4.14 ± 0.02[a]	3.97 ± 0.05[b]
8	4.00 ± 0.03	3.88 ± 0.04
9	3.85 ± 0.03[a]	3.75 ± 0.02[b]
10	3.73 ± 0.02	3.62 ± 0.05
11	3.61 ± 0.02	3.55 ± 0.05
12	3.46 ± 0.07	3.43 ± 0.06
13	3.34 ± 0.06	3.31 ± 0.03
14	3.17 ± 0.03	3.24 ± 0.05
15	3.05 ± 0.03	3.16 ± 0.05
16	2.96 ± 0.02	3.07 ± 0.07
17	2.88 ± 0.04	2.99 ± 0.04
18	2.80 ± 0.05	2.91 ± 0.03
19	2.67 ± 0.03[a]	2.81 ± 0.04[b]
20	2.61 ± 0.04	2.73 ± 0.03
21	2.51 ± 0.06	2.65 ± 0.04
22	2.44 ± 0.04	2.55 ± 0.04
23	2.39 ± 0.05	2.49 ± 0.05
24	2.33 ± 0.03	2.43 ± 0.05
25	2.26 ± 0.02	2.33 ± 0.06
26	2.15 ± 0.01	2.25 ± 0.06
27	2.10 ± 0.02	2.18 ± 0.05
28	2.04 ± 0.02	2.10 ± 0.04
29	1.93 ± 0.05	1.88 ± 0.10
X	5.70 ± 0.10	5.45 ± 0.09
Y	1.77 ± 0.10	-

[a,b] Indicates values within row differ significantly (P<0.05).

(The metaphase plate from male and female Tho-Tho cattle are presented in Plates 1 and 2). The significant results are; (i) autosomes in both sexes of Tho-Tho cattle are acrocentric, (ii) relative length (mean ± SE) of the autosomes ranges from 1.93 ± 0.05 to 5.19 ± 0.12% in case of male and 2.10 ± 0.04 to 5.38 ± 0.16% in case of female, (iii) karyotype of the female Tho-Tho cattle has two large submetacentric ‘X’ chromosome, whereas the male Tho-Tho cattle has one large submetacentric ‘X’ chromosome and a small acrocentric ‘Y’ chromosome, (iv) ‘X’ chromosome contributes 5.70 ± 0.10% in male and 5.45 ± 0.09% in female, which are larger than the first pair of autosomes, (v) ‘Y’ chromosome contributes 1.77 ± 0.10% towards the total haploid genome in male and it was the smallest complement of male karyotype, (vi) length of first autosome pair is 5.19 ± 0.12% in male and 5.38 ± 0.16% in female, whereas the twenty-ninth smallest autosomes contributes 1.93 ± 0.05% in male and 1.88 ± 0.10% in female respectively and (vii) the centrometric index of ‘X’ chromosome (Figure 1) was 33.75% in male, but in case of female it was 32.76% (Viii) while the arm ratio (Figure 1) of “X” chromosome was 0.66 in case of male, it was 0.67 in case of female Tho-Tho cattle.

Statistical analysis showed Tho-Tho cattle population amounts to number 175897 which is 39% of the total indigenous cattle of the state of Nagaland. The study area which constitutes 3 districts has a population of Tho-Tho cattle of 37000 which is 5.33% of the total cattle population of the state. Analysis of cytogenetic characters revealed that the relative length of 23 pairs of autosomes did not differ significantly (P < 0.05) between male and

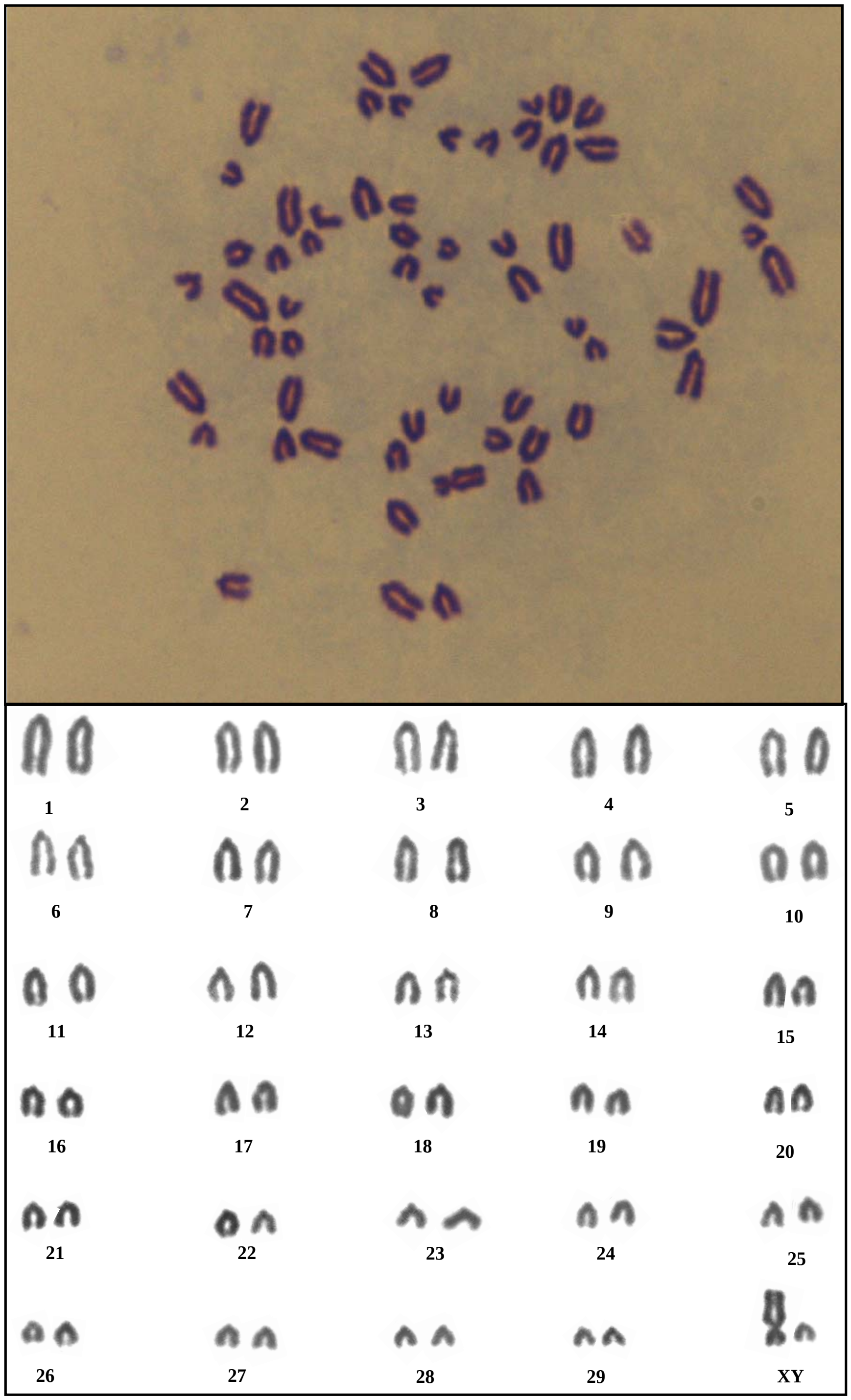

Plate 1. Karyotype of a male Tho-Tho cattle.

female Tho-Tho cattle. The relative length of 4th, 5th, 7th, and 9th number of autosomes were significantly ($P < 0.05$) higher in male (4.53 ± 0.04%, 4.42 ± 0.06%, 4.14 ± 0.02% and 3.85 ± 0.03%) than that in female (4.34 ± 0.04%, 4.22 ± 0.05%, 3.97 ± 0.05% and 3.75 ± 0.02%). But the relative length of 19th number autosome was found to be significantly ($P < 0.05$) higher in female (2.81 ± 0.04%) than that in male (2.67 ± 0.03%). The relative length of X-chromosomes were 5.70 ± 0.10% and 5.45 ± 0.09% in male and female Tho-Tho cattle respectively and the difference was non-significant ($P < 0.05$). The centromeric index and the arm ratio were also non-

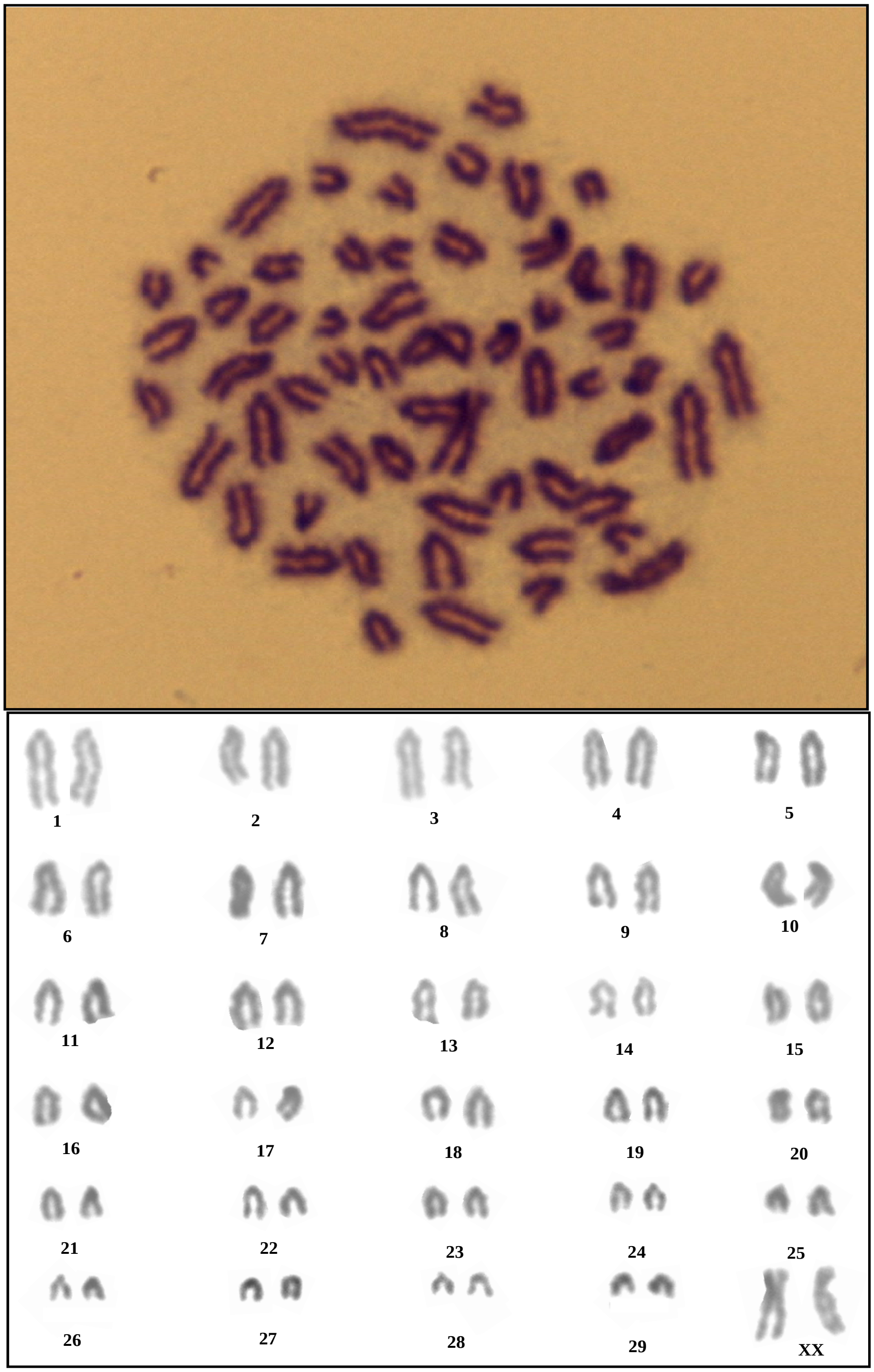

Plate 2. Karyotype of a female Tho-Tho cattle.

significant between male and female Tho-Tho cattle.

DISCUSSION

The karyotype analysis of Tho-Tho cattle revealed a diploid (2n) chromosome number in all the complete metaphase plates. All the 29 pairs of autosomes in both sexes of Tho-Tho cattle were found to be acrocentric. The larger chromosomes were distinguishable from the smaller ones but the decrease in size was so gradual that further sub-classification was not possible (Plates 1 and 2). Similar results are also reported by Yadav (1981) in Sahiwal, Tharparkar, brown Swiss, Holstein Friesian and

Jersey, Sarkhel (1988) in Gir, Jersey and Holstein Friesian, Nivsarkar et al. (1992) in Tharparkar, Kumar et al. (1995) in Hariana, Vijh et al. (1996) in Rathi, Vij et al. (1997) in Nagori, Choudhury et al. (1997) in Assam local cattle and Dayananda (2000) in Nimari breeds of cattle.

The morphology of sex chromosomes is a distinguishing factor differentiating *Bos indicus* from other species. The cattle chromosome can easily be distinguished from that of goat as the later has an acrocentric ‘X’ and metacentric ‘Y’ chromosome. This can further be characterized by small acrocentric ‘Y’ chromosome which is not found in *Bos taurus*, *Bos banteng* (Bali cattle), *Bos grunniens* (Yak), and *Bos mutus* (Wild yak). Sarkhel (1988) reported that the crossbred males from exotic sire have a small submetacentric ‘Y’ chromosome. Absence of the small submetacentric chromosome revealed the purity of Tho-Tho cattle as an indigenous breed of the country. The results of the present study in respect of relative length are in close agreement with Sarkhel (1988), Vij et al. (1997) and Choudhury et al. (1997) in Gir, Hariana, Nagori and Assam local cattle respectively. Although, it was difficult to identify the exact position of ‘Y’ chromosome in the karyotype without banding, the smallest acrocentric chromosome of the chromosomal complement was considered as ‘Y’ chromosome, based on the reports of earlier studies by Gupta et al. (1974), Yadav (1981) and Sarkhel (1988) in different breeds of *Bos indicus* cattle and the absence of smaller meta/sub-metacentric chromosome. However, Vij et al. (1997) reported that the ‘Y’ chromosome should be placed between 27th and 28th autosomes o f the karyotype.

The values of centromeric index are also in close agreement with that of Yadav (1981), Sarkhel (1988), Vijh et al. (1996) and Dayananda (2000) in different indigenous breeds of cattle.

The Tho-Tho cattle belongs to an indigenous breed (*Bos indicus*) which can be characterized by the chromosome number, morphology of autosomes and sex chromosome. The unique relative length of chromosome determines its similarity with other indigenous breeds of India. However, complete characterization is possible only after comparison with many other indigenous breeds and different types of bandings (G-banding, C-banding and High resolution banding), which may reveal the exact structure of chromosome specific for the breed.

Conclusion

The experimental findings reveals that the cytogenetic characteristics of Tho-Tho cattle are at par with other high yielding indigenous breeds of cattle. There are also some positive traits in Tho-Tho cattle such as (i) resistance to disease, (ii) tolerance to extreme weather conditions, (ii) draught power, (iv) good carcass quality, and (v) efficient conversion of low quality feeds. Tho-Tho cattle play a crucial role in the livelihood of the small farmers. Therefore, the extension and institutional systems should take necessary steps to conserve, propagate and upgrade this valuable indigenous germplasm. Suitable breeding strategies of Tho-Tho cattle and farmers’ awareness programme are also required for development of cultural, socio economic, dairy, draught and agricultural sector of Naga people.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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