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PHYSICAL SEMEN CHARACTERISTICS AND SPERM DNA INTEGRITY OF APRI LINE AND BALADI BLACK RABBIT BUCKS IN DIFFERENT SEASONS

Shahira, M.M. El-Gganainy[1]; Ayat, A. Ragab[2]; A.M. Shaarawy[2] and A. Omar[2]

[1]Animal Production Department, Faculty of Agriculture, Minia University.

[2]Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt.

Corresponding author: shahira.elganainy@mu.edu.eg.

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SUMMARY

The aim of the present study was to evaluate the seasonal variations in sperm quality of APRI Line and Baladi Black Rabbit Bucks kept under Egyptian climate. Semen ejaculates from 20 rabbit bucks of APRI and Baladi Blacks (BB) in winter, spring, autumn and summer were analyzed. Sperm quality parameters such as kinematics, morphology and DNA integrity were analyzed and study the effect of climate factors recorded by the web of local meteorological office. There were significant differences between ARRI and BB for ejaculate volume (0.59, 0.55 ml), live sperm per ejaculate (83.54, 79.34%), progressive motility (58.00 and 60.00 %), comet length (37.06 and 38.34 px) comet height (34.18 and 37.74 px) and head diameter (33.54 and 36.53 px), respectively. Whereas, non-significant differences were observed due to the effect of line on semen pH, sperm abnormalities per ejaculate, tail length and DNA % in tail. The effect of season was significant for ejaculate volume (0.64 and 0.48ml), live sperm per ejaculate (84.10 and 78.20 %), progressive motility (76.00 and 45.00%), sperm abnormalities per ejaculate (11.52 and 16.20%), comet length (36.64 and 38.76), tail length (2.55 and 5.06 px) and DNA% in tail (6.17 and 4.26%) during winter and summer seasons, respectively while, non-significant differences were observed between seasons for semen pH, comet height and head diameter. The results showed that the highest ejaculate volume and live sperm per ejaculate were recorded during winter with APRI moreover; sperm abnormalities per ejaculate in summer season were lower for APRI (14.60 %) than for BB (17.80 %). It is concluded that, APRI line rabbit bucks tended to have larger ejaculate volume, lower semen pH, higher total live sperm, higher progressive motility percentage and lower abnormal percentage than those displayed by the BB rabbit bucks this, could be an indicator to an acceptable line that performs well under Egyptian conditions.

Keywords: *Rabbit line, Climate conditions, Semen quality, Sperm DNA.*

INTRODUCTION

Rabbit is the most recommended animal to facilitate the meat shortage problem in Egypt. Hence, rabbit is a good quality meat and respond well to genetic improvement through selection. Despite the gradual increase in rabbit productivity in Egypt, rabbits still face numerous genetic and environmental problems such as climatic factors occasioned by high temperature (Farghly *et al.,* 2020) and heat stress (Huang *et al.,* 2023). The environmental temperature is the largest stressor affecting the efficiency of animal production and reproduction systems of all animals (Shahat *et al.,* 2020). In fact, a vital factor for survival among male rabbit is reproduction (Ousmane *et al.,* 2019; Eliraqy *et al.,* 2024) that closely associated to the production of semen containing a high proportion of normal spermatozoa. A common problem for rabbit breeder especially in dry hot countries is unfavorable weather conditions and heat stress (Ousmane *et al.,* 2019) causing reduced sperm motility and increased abnormalities along with poor fertility. Spermatozoa are highly specialized cells that suspended in seminal plasma and control fertility and embryo development (Castellini, 2008). The assessment of sperm quality parameters in the laboratory like morphological features, motility, acrosome status, plasma membrane integrity, and metabolic activity is a convenient indirect way of detecting alterations in a male's ability to reproduce, without conducting an artificial insemination (AI) trial on each ejaculate (Sabés-Alsina *et al.*, 2019). Therefore, Drobnis *et al.,* (2015) stated that sperm DNA integrity test is usually assessed as highly predictive index of reproductive outcome that correlated with success fertilization and normal development of the embryo and offspring (Zini, 2011; Palermo *et al.,* 2014). All of these sperm characteristics are susceptible to extraneous factors such as climate, level of nutrition, genotype, individual traits and management practice (Snoj *et al.*, 2013; Bhakat *et al.,* 2014; Stádník *et al.,* 2014; Zuzana Biniová *et al.,* 2017), as well as intrinsic factors such as age, the collection frequency, lighting programs, health, and feeding strategies (Snoj *et al.,* 2013; Bhakat *et al.,* 2014; Stádník *et al.,* 2014).

One of the most important factors that affect semen quality is buck breed (Alragubi, 2015). Baladi Black (BB) rabbits, an Egyptian meat-type breed characterized by their high tolerance to climatic stress and their resistance to disease (Khalil et al 2014, were originated by crossing heavy Baladi does with pure Giant Flander bucks, through the selection of heavy body weight and pure black color traits over several generations (Meshreky *et al.*, 2005). Egyptian researchers used to select rabbit breeds to produce lines more adapted to calamite under Egyptian environmental conditions (e.g. The APRI line). The APRI line is a cross between Baladi Red bucks with V-line does to produce F1 (½B½V) stock, followed by two generations of inter se mating to achieve performance stability (Youssef *et al.*, 2008; Abou Khadiga *et al.*, 2010a, b). It seems that this rabbit line successfully adapted to the local environmental conditions and superior reproductive performance scale on an intensive level (Abou Khadiga *et al.*, 2012).

It is well known that, most of the sperm disorders are belonging to elevating ambient temperate and increasing the relative humidity. Till now, there has been insufficient information about the overall semen quality of APRI bucks in different seasons in Egypt.

The aim of this study was to evaluate some semen characteristics of APRI and local breed Baladi Black bucks under Egyptian different season conditions.

MATERIALS AND METHODS

Climatic conditions:

During different seasons, the maximum and minimum air temperatures (T ºC) and relative humidity (RH %) were obtained from Egyptian Meteorological Authority web site, then temperature-humidity index (THI) values were calculated (Table 1).

Table (1): Means maximum and minimum air temperature (ºC), the percentage of relative humidity (%) and calculated values for the temperature-humidity index during the experimental period.

Month	Air temperature			Relative humidity%	Temperature-humidity index
	Maximum	Minimum	Average		
December	22	14	20	72	66.43
January	26	12	20	45	64.92
February	29	12	22	50	67.80
March	31	17	26	63	74.51
April	35	15	29	50	76.90
May	34	23	32	50	80.80
June	39	27	34	57	84.77
July	37	24	33	60	83.96
August	40	26	36	55	87.08
September	38	24	34	66	86.54
October	35	25	33	65	84.89
November	27	20	24	70	72.32

The THI was calculated using the formula reported by Garcia-Ispierto et al. (2006) as the following:

$$THI = 0.8 * \text{ Mean T} + \frac{\text{Mean RH}}{100} * (\text{ Mean T} - 14.4) + 46.4$$

The calculated values of the THI were classified as follows: below 72 is no heat stress, from 72.1 to 83 is moderate heat, from 83.1 to 86 is severe heat stress, above 86 very severe heat stresses.

Animals management and semen collection:

Semen ejaculates were collected from 20 rabbit bucks (10 APRI and 10 BB) at 6 months of age (sexually mature) kept at Sakha experimental station rabbitry, Kafr El-sheikh Governorate belonging to Animal Production Research Institute, Egypt during winter (December, January, and February), spring (March, April, and May), summer (June, July, and August), and autumn (September, October, and November). Semen ejaculates were regularly collected twice/week according station schedule during these times using an artificial vagina as described by Mocé et al. (2000) for 10 weeks per season winter (1 December to 28 February)spring (7 March to 31May), summer (7 June to 31 August), autumn (7September 1 to 30 November).

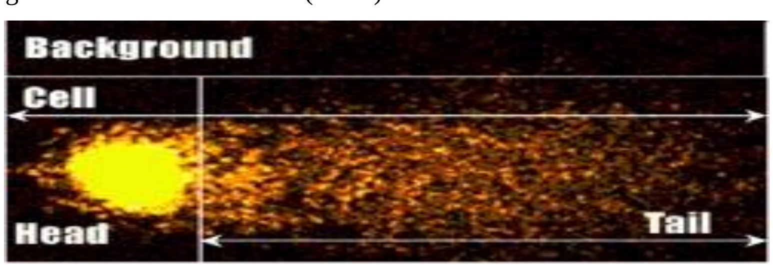
All rabbit bucks were healthy and housed in individual cages. A constant supply of fresh tap water was automatically available via stainless steel nipples located inside each cage. Rabbits were fed a commercial pelleted diet ad-libtum (16.40% CP, 12.70 % CF and DE 2403 kcal/kg) that covers their nutritional requirements in all phases according to NRC (2001) recommendations.

Semen analysis:

The raw ejaculate was evaluated manually and examined under a phase-contrast microscope (Leica DM 500, Leica, Germany). Semen volume was measured per ml by using a graduated tube. Net semen volume was recorded for the ejaculates containing gel (El-Sherbiny, 1987). The pH value of the semen samples was measured directly after collection using comparative paper (Whatman pH Indicator paper, Whatman Limited Maidstone, England). Percentage of live spermatozoa was estimated using eosin-nigrosin stain (1.67 g eosin and 10 g nigrosin in 100 ml of 2.90 % sodium citrate buffer) according to (WHO, 1999). Live spermatozoa were not stained and head was appeared as bright cells while, dead spermatozoa were stained and head appeared in pink color. Percentage of progressive motility was estimated using semen diluted with physiological saline 3% sodium citrate kept on a warm slide and covered with cover slip each sperm sample was examined in 10 microscopic fields at x100 magnification (Gamal *et al.,* 2016). The morphological abnormalities (head, mid-piece and tail defects) of spermatozoa were determined in the same slide smears, which prepared for live/dead ratio under light microscope at 100x magnification counting at least 100 sperm per sample. The total number and percentage of abnormal spermatozoa were calculated by using 2:3 microscopic fields for each slide.

Sperm DNA damage:

The method used to evaluate DNA damage was a comet assay "single-cell gel Electrophoresis" accords to Fraser and Strzezek (2005). The head region of a comet: sperm cells represent un-fragmented DNA that does not migrate outside the region of the nucleus. Tail length: sperm cells represent DNA that migrates due to fragmentation and loss of structure. At higher degree of damage, the tail length becomes steady whereas the amount of DNA migrating into the tail still increases (Pic. 1).



Pic. 1. Scoring of DNA damage in head and tail of sperm cell.

Statistical analysis:

Data of semen characteristics were analyzed by factorial design using SAS software, SAS (2004) to set the effect of line (1-2), season (1-4), and their interaction based on the following model:

$$Yijk = \mu + l_i + S_j + (lS)_{ij} + e_{ijk}$$

Where: μ = Overall mean, l_i= Effect of the line, S_j = Effect of the season, $(lS)_{ij}$ = The interaction between the line and the season, and e_{ijk} = Random error.

The differences among means were tested using Duncan's Multiple Range Test (Duncan, 1955) at a level of $P<0.05$.

RESULTS AND DISCUSSION

Climatic conditions during different months:

As presented in Table 1, the maximum air temperature was recorded during the summer season, ranged from 37 to 40ºC and the corresponding THI values varied from 83.96 to 87.08. Hence to bucks were exposed to sever and very sever heat stress in this season. However, the minimum air temperature in winter season was 12 ºC and the mean THI was 64.92 that defined as the non-heat stress season. the obtained data accordance with Marai et al. (2002) who found that severe heat stress occurs when air temperature is over than 28.9o C or THI than 87.30 (Safaa *et al.*, 2008).

The physical semen parameters:

Sperm characteristics in APRI and (BB) rabbit bucks as affected by rabbit line, season, and their interaction are presented in Table 2.

Semen ejaculates volume (ml):

Average of net semen volume was significantly higher in APRI than in BB. El-Tarabany *et al.* (2015) and Jimoh and Ewuola (2019) indicated that, breed had significant effect on ejaculate volume. Also, Abd El-Azim and El-kamash (2015) stated that under the environmental conditions of Egypt the New zland rabbit was superior in semen ejaculate volume than Balady rabbit. As affected by season, net semen volume was significantly ($P<0.05$) difference the highest in winter, moderate in spring and autumn, and the lowest in summer. However, Iraqi *et al.* (2012) noted that season of the year had no significant effects on semen ejaculate volume. Also, the effect of interaction between line and season on net semen volume was significantly difference, indicating the highest values in winter and the lowest values in summer for both lines, being higher in APRI than in BB (Table 2). The variation in the ejaculate volume between lines during different seasons may be due to differences in the rate of the accessory sex glands activity in response to testosterone concentration (Iraqi *et al.*, 2012). In this respect, several authors indicated the lowest buck's ejaculate volume in summer (Hosny *et al.*, 2020; Huang *et al.*, 2023). The observed decrease in net semen volume in summer may be attributed to the negative effect of high temperature on the level of testosterone production which was decreased by increasing the ambient temperature (Abu Zeid, *et al.*, 2017).

Semen pH value:

The average semen pH values weren't significant differences between APRI and BB. As affected by season semen pH values were ($P>0.05$) highest in summer, moderate in autumn, and lowest in spring and winter. Previously, Huang et al. (2023) noted non-significant effect of heat stress on semen hydrogen ion concentration. Moreover, semen pH values were ($P>0.05$) highest in summer for BB line while, lowest values were in winter for APRI line (Table 2). Seminal plasma usually is an isotonic neutral medium and it is a key factor to sperm cell survival (White, 1976). Measurement of semen pH is great importance due to any semen extender used, it should act as a buffer against excessive acidity or alkalinity and also, it acts as indicator for the normal accessory glands secretion and the livability of spermatozoa (Jimoh and Ewuola, 2019).

Live sperm percentage per ejaculate:

There was a significant difference ($P<0.05$) between the two lines in live sperm % per ejaculate. Also, there were significant differences ($P<0.05$) among seasons being higher in winter, moderate in spring and autumn, and lower in summer. This concurs with the results of Hosny et al. (2020) and Huang *et al.* (2023), who reported a significant adverse effect of heat stress on live sperm percentage in rabbits. The highest live sperm percentage per ejaculate was recorded during the winter season with APRI line the lowest in summer with BB breed (table 2). The increase in dead sperm percentage in semen of bucks reared in summer may be due to spermatozoa susceptibility to oxidative stress that increases during heat stress (Ganaie *et al.*, 2013). In addition, this may be due to the negative effect of heat stress on testosterone which control epididymis function (Abu Zeid *et al.*, 2017 and Hosny *et al.*, 2020).

Sperm motility percentage:

The average progressive motility percent was significant higher in BB than APRI line. Khalil *et al.* (2014) and Jimoh and Ewuola (2019) reported that the breed has significant effect on motility of sperms, the sperm activity degree importance for passing through the oviduct and completing fertilization. Also, as affected by season, spermatozoa motility% was significantly ($P<0.05$) higher in winter than in summer. These results were in agreement with, El-Ratel *et al.* (2021); Farghly *et al.* (2020). Huang *et al.* (2023) reported that the sperm motility decreased significantly ($P<0.01$) in hot than during cold season.

The higher percentages of progressive motility observed in samples during winter season with both line (BB and APRI) than summer season (Table 2). Hosny *et al.* (2020) when rabbit exposed to heat stress the testicular activity was negatively affected, testosterone secretion depressed and consequently, sperm quantity and quality (motility%, abnormal %) of sperm reduced.

Table (2): Least square mean and standard error (LSM±SEM) of semen characteristics in APRI and Baladi Black (BB) rabbit bucks during different seasons of year.

Item	N	Net semen volume (ml)	Semen pH value	Live sperm (%)	progressive motility (%)	Sperm abnormality (%)
Effect of buck line (B):						
APRI	80	0.59[a]	6.96	83.54[a]	58.00[b]	12.93
BB	80	0.55[b]	6.99	79.34[b]	60.00[a]	14.93
SEM		0.01	0.03	1.38	1.87	0.92
Effect of season (S):						
Winter	40	0.64[a]	6.94	84.10[a]	76.00[a]	11.52[b]
Spring	40	0.58[b]	6.95	82.23[ab]	65.00[b]	13.13[ab]
Summer	40	0.48[c]	7.00	78.20[b]	45.00[c]	16.20[a]
Autumn	40	0.58[b]	6.98	81.24[ab]	51.00[c]	14.68[ab]
SEM		0.02	0.03	1.94	2.25	1.31
Interaction Effect (LxS):						
APRI*winter	20	0.66	6.92	86.10	75.00	10.90
APRI*spring	20	0.59	6.93	84.25	64.00	12.90
APRI*summer	20	0.52	6.98	80.30	44.00	14.60
APRI*autumn	20	0.60	6.97	83.52	49.00	13.30
BB*winter	20	0.61	6.97	82.10	78.00	12.15
BB*spring	20	0.57	6.98	80.20	66.00	13.35
BB*summer	20	0.45	7.02	76.10	46.00	17.80
BB*autumn	20	0.56	7.00	80.00	53.00	16.05
SEM		0.03	0.05	2.75	3.18	1.85

a,b,c… Means in the same column for each effect with different superscripts are significantly different at $P<0.05$.

The percentage of sperm abnormalities per ejaculate:

No significant differences in the percentage of sperm abnormality were found between APRI and BB lines. El-Darawany and El-Sayied, (1994) and Daader *et al.* (1999) found that there were non-significant differences in percentages of abnormal spermatozoa among different rabbit's breeds, however, other investigator reported that there were significant differences in normal spermatozoa due to breeds (Abd El-Azim and El-Kamash, 2015). The percentage of sperm abnormalities was being higher in summer and moderate in spring and autumn, and lower in winter (Table 2). Obtained results are in accordance with; Safaa *et al.* (2008), Alvariňo (2000) and Iraqi *et al.* (2012) who recorded the highest percentage of sperm abnormalities per ejaculate were in hot season as compared with other seasons. Regarding to interaction between line and season on percentages of abnormal spermatozoa the lowest percent in winter season was recorded for APRI line and the highest for BB in summer. This indicates that, APRI has more adaptation with hot climate conditions than BB for abnormalities spermatozoa this could be due to genetic progress by crossing Baladi Red bucks with V-line does (Abou Khadiga *et al.,* 2012). This may be attributed to the deleterious effect of the hot weather months on the normal spermatozoa of rabbit semen (Nasr, 1994; Meshreky and Abbas, 2000 and Seleem *et al.,* 2010). It is important to mention that the presence of large number of abnormal spermatozoa in semen decreases its fertility (Iraqi *et al.,* 2012). The present results showed that hot season had a negative effect on sperm motility, concentration and its viability this result are agreement with findings Hamerezaee *et al.* (2018) who reported that considerable reduction in sperm count viability along with poor fertility however, chromatin irregularities was increased with exposure to heat stress.

Sperm DNA fragmentation:

There were significant differences (P<0.05) between the two studied lines in comet length, comet height and head diameter and non-significant differences (P>0.05) in tail length and DNA in tail %. Moreover, obtained results showed that there was significant effect of summer season conditions on comet length, tail length and DNA in tail% and non-significant differences (P<0.05) in comet height and head diameter (Table 3). It was evident that APRI line exposed to high temperature during summer season can induce increase DNA damage through increasing DNA in tail and tail length. This result is in agreement with findings with Hamilton et al. (2018) who concluded that heat stress had a vital effect on sperm DNA breakup of adult rams. Moreover, Pérez-Crespo et al (2008) reported that heat stress induces negative changes on sperm DNA this to increasing testicular temperature that negatively affect normal sperm morphology and spermatogenesis.

Table (3): Least square mean and standard error for comet assay of sperm DNA variables (LSM±SEM) in APRI and BB rabbit bucks during the different seasons.

Traits / Classification	No. of observations	Comet Length (px)	Comet Height (px)	Head diameter (px)	Tail Length (px)	DNA in tail %
Effect of buck line (B):						
APRI	219	37.06^{b}	34.18^{b}	33.54^{b}	3.91	5.54
BB	219	38.34^{a}	37.74^{a}	36.53^{a}	3.71	4.88
SEM ±		00.37	00.70	00.68	00.32	00.54
Season (S):						
Winter	249	36.64^{b} ±0.68	36.17 ±0.63	35.32 ±0.63	2.55^{b} ±0.34	6.17^{a} ±0.50
summer	189	38.76^{a}±0.78	35.74 ±0.72	35.32 ±0.72	5.06^{a} ±0.30	4.26^{b} ± 0.57
Interaction Effect (LxS):						
APRI* Winter	124	30.68	30.00	29.16	1.52	6.61
BB* Winter	125	34.09	33.10	32.72	3.82	4.05
SEM±		1.10	1.03	1.02	0.42	0.81
APRI* summer	95	43.44	38.38	37.93	6.31	4.48
BB* summer	94	42.60	42.37	40.33	3.59	5.72
SEM±		0.96	0.89	0.89	0.49	0.71

a,b,c… Means in the same column for each effect with different superscripts are significantly different at P<0.05.

Pic.2. Fluorescence microscopy displaying spermatozoa of DNA damage

CONCLUSION

Based on the current results, it appeared that the semen characteristics of APRI line buck rabbits presented better semen quality than those of the BB buck rabbits under environmental condition in Egypt. On the contrary, heat stress induces DNA damages of APRI rabbit spermatozoa.

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الخصائص الفيزيائية للسائل المنوي وسلامة الحمض النووي للحيوانات المنوية لدى ذكور الأرانب الابري والبلدي الاسود خلال المواسم المختلفة

شهيرة محمد محمود الجنايني[1]، آيات عبد المقصود رجب[2]، المعتز بالله محفوظ شعراوي[2] و امال عمر[2]

[1]قسم الانتاج الحيواني – كلية الزراعة – جامعة المنيا – المنيا - مصر

[2]معهد بحوث الانتاج الحيواني – مركز البحوث الزراعية – الدقي – مصر

الهدف من هذة الدراسة معرفة مدي تاثير الظروف المناخية الموسمية المختلفة علي جودة الحيوانات المنوية لذكور الأرانب (الابري APRI والبلدي ال اسودBB) التي يتم تربيتها تحت الظروف الجوية في مصر. تم تحليل عينات السائل المنوي من 20 ذكرًا من الأرانب من سلالة الابري APRIوالبلدي الاسود BBخلال موسم الشتاء والربيع والخريف والصيف. تم تحليل معايير جودة الحيوانات المنوية مثل الحركة وتشوهات الحيوان المنوي وسلامة الحمض النووي وربطها بعوامل المناخ المسجلة من قبل مكتب الأرصاد الجوية المحلي. كانت هناك فروق ذات دلالة إحصائية بين ARRI وBB لحجم القذف (0.59، 0.55 مل)، والحيوانات المنوية الحية لكل قذفة (83.54، 79.34٪)، والحركة الفردية (58.00 و60.00٪)، وطول المذنب (37.06 و38.34 بكسل) وارتفاع المذنب (34.18 و37.74 بكسل) وقطر الرأس (33.54 و36.53 بكسل)، على التوالي. في حين لوحظت فروق غير معنوية بسبب تأثير السلالة على درجة حموضة السائل المنوي وتشوهات الحيوانات المنوية لكل قذفة وطول الذيل ونسبة الحمض النووي في الذيل. كان تأثير درجة حرارة الهواء (درجة مئوية) معنويًا لحجم القذفة (0.64 و0.48 مل) والحيوانات المنوية الحية لكل قذفة (84.10 و78.20٪) والحركة الفردية (76.00 و45.00٪) وتشوهات الحيوانات المنوية لكل قذفة (11.52 و16.20٪) وطول الذيل (2.55 و5.06 بكسل) ونسبة الحمض النووي في الذيل (6.17 و4.26٪) خلال فصلي الشتاء والصيف على التوالي، بينما لوحظت فروق غير معنوية بين المواسم لدرجة حموضة السائل المنوي وارتفاع المذنب وقطر الرأس. أظهرت النتائج أن أعلى حجم قذفة وحيوانات منوية حية لكل قذفة تم تسجيلها خلال فصل الشتاء مع سلالة ال APRI علاوة على ذلك؛ كانت التشوهات في الحيوانات المنوية لكل قذفة في موسم الصيف أقل في سلالةAPRI 14.60٪ مقارنة بسلالة BB 17.80٪.

وتوصلت الدراسة إلى أن ذكور الأرانب من سلالة APRI تميل إلى أن يكون لديها حجم قذف أكبر، ودرجة حموضة أقل للسائل المنوي، وإجمالي حيوانات منوية أعلى، ونسبة حركة أعلى ونسبة تشوهات أقل من تلك التي أظهرتها ذكور الأرانب من سلالة BB، وهذا يمكن أن يكون مؤشرًا على انها سلالة مقبولة تؤدي أداءً جيدًا في ظل الظروف المصرية.