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Effects of moisture stress at flowering on phenotypic characters of selected local maize landraces in Kenya

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1 SUMMARY

Arid and semi arid areas constitute about 82% of the total land area and supports about 20% of Kenya's human population. Local maize landraces are an important livelihood resource in these areas. The objective of the study was to characterize selected Kenyan maize landraces for drought tolerance. Secondary traits exhibiting high heritability for drought tolerance such as grain yield, anthesis-silking interval (ASI), tassel size, ears per plant and leaf rolling were evaluated. In season I, the 25 genotypes were grown under optimal conditions (under normal rainfall supplemented with irrigation) for the determination of the anthesis-silking interval (ASI), ears/plant, tassel size and grain yield. In season II, based on the ASI the genotypes were planted in an Alpha lattice design in two separate experiments; optimum and water stressed conditions each replicated 3 times. In the water stressed plots, irrigation was withheld one week to tassel anthesis and resumed after male flowering had been achieved. Among the characters evaluated, a low ASI (1-6 days) was associated with a high level of drought tolerance and low yield losses. Drought stress resulted in 17 to 81% relative grain yield loss. Landraces GBK-032419 and GBK-034659 exhibited lowest grain yield losses of 28 and 17%, respectively, while two dry-land Composites, Katumani Composite B (KCB) and Makueni Dryland Composite (DLC) used as controls exhibited higher grain yield losses of 62 and 68%, respectively. In general, an increase in the number of ears per plant, 100-seed weight, increased plant height, reduced leaf rolling and low ASI were associated with yield increases under moisture stress. Local landraces that exhibit drought tolerance were identified. Drought tolerant landraces included GBK-034659, GBK-032419, GBK-044593, GBK-032423 and GBK-027054. These could be recommended for production in marginal areas of Kenya. Research to further stabilize yields in these landraces could play a key role in mitigating hunger in Kenya. Drought tolerance traits identified could be introgressed into recommended Composites for the marginal areas.

2 INTRODUCTION

Only about 18% of Kenya's land area is classified as medium to high potential agricultural land, but the remaining 82% that is arid to semi arid has to play a major role in providing sources of livelihoods to many Kenyans (Government of Kenya, 2004). In Kenya, maize is the most important staple food crop grown on about 1.6 million hectares of land (FAO, 2007). The average maize yields of highland farmers in Kenya range from 2.8 to

3.5 t ha^{-1} though some achieve 7.0 t ha^{-1} while that for low altitude farmers is only 1.0 t ha^{-1} (FAO, 2007). The challenge to crop production in dry land areas is compounded by the fact that the occurrence, timing and severity of drought fluctuates from year to year (Ribaut *et al*., 2002). The drought problem can be solved through provision of irrigation water and / or technologies such as drought tolerant cultivars that withstand water stress. However, irrigation is becoming a lesser solution as global water demand increases (Boyer and Westgate, 2004). In many marginal areas farmers use local landraces because they are perceived to perform better under low moisture and/no input use (Bellon *et al*., 2006). Some local maize landraces exhibit desirable attributes like resistance/tolerance to major diseases and pests, tolerance to drought and productivity under low soil fertility (Mugo *et al.,* 1998). The existence of these highly valuable maize landraces represents a potential wealth of genetic material already adapted to widely varying environments. Secondary traits are few plant characteristics that are highly heritable and whose variation results in proven genotype x environment interactions for grain yield. They are important in maize characterization for drought tolerance as they improve the precision with which drought tolerant germplasm can be identified (Banziger *et al*., 2000). Further, secondary traits are correlated with yield and demonstrate segregation with high heritability under water-limited conditions (Ribaut *et al*., 2002). Though several hybrids have been developed, they have failed to produce satisfactorily in the dry areas. Many farmers therefore continue to grow local landraces, which are perceived to perform better under drought stress and low input conditions. While the existence of local maize landraces is recognized, only a few have been characterized for drought tolerance. The objective of this study was to evaluate the effects of moisture stress at flowering on selected maize landraces using secondary traits.

3 MATERIALS AND METHODS

3.1 Experimental site: The experiments were carried out at the Kenya Agricultural Research Institute (KARI) Masongaleni farm, Kibwezi district, Eastern province, Kenya. The farm is located at 2° 21.6' South and 38° 7.3' East, and about 650 meters above sea level. The farm is within the semi-arid areas of Kenya, agro-ecological zone VI and receives approximately 400mm of rainfall per year with an annual evapotranspiration potential of between 1650-2300mm. The soils are rhodic and orthic *Ferrasols* (Jaetzold and Schmidt, 1983).

3.2 Germplasm: The experimental materials consisted of 25 germplasm, 15 of which were local maize landraces from the Gene Bank of Kenya (GBK). Ten inbred lines were included for comparison purposes. These included 5 lines from KARI-Kitale, 3 from KARI-Katumani and 2 from CIMMYT-Nairobi. Katumani Composite B and Makueni Dry Land Composite (DLC) which are recommended for these marginal areas were used as checks.

3.3 Experiment set up: In season I which was conducted between February and May 2006, the germplasm was grown under optimum conditions under normal rain supplemented with irrigation for the determination of their anthesis-silking interval (ASI), ears/plant, tassel size and grain yield. Experiment was set up in Alpha Lattice design and inbred lines were randomized together in same blocks to avoid shading. The crop was protected from pests and weeds so that characters could be expressed well. Bifenthrin 25g/L (Brigade) insecticide was sprayed three days after seedlings emergence to protect against insect pests damage and the field was kept weed free through hand hoeing. The crop was top dressed with Calcium Ammonium Nitrate (CAN) at the rate of 48kg N per ha 30 days after planting. Trichlorfon 2.5% (Dipterex) granules were applied when seedlings were about 45cm high to protect against stalk borer attacks. Diammonium Phosphate (DAP) fertilizer was applied at planting at the rate of 40kg/ha N and 102 kg/ha P_2O_5 (Ministry of Agriculture, 1987). Each experimental plot consisted one row of ten plants spaced at 0.75m x 0.3m. Data was collected from the 8 middle plants, discarding the first and last plant on each row.

In June 2006, based on the ASI genotypes with close or equal days to tassel anthesis were grouped together for synchronized planting for easier management of stress conditions during flowering (irrigation withdrawal at flowering stage). All the genotypes were therefore expected to reach tassel anthesis at approximately the same time. They were planted in an Alpha lattice design in two separate experiments; optimum and water stressed conditions each replicated 3 times. Each experimental plot was 2.25m x 2.7m in size. Husbandry practices were executed as described in experiment one above. In drought stressed plots irrigation was withdrawn one week to tassel anthesis. Irrigation resumed later when male flowering had been achieved. The well-watered plots had sufficient water throughout to ensure normal maize plant growth. The germplasm description and corresponding ASI are given in Table 1.

3.4 Data recording and analysis: Data taken included days from sowing to 50% tassel anthesis (AD), days from sowing to 50% silking (SD), anthesis-silking interval (ASI), leaf rolling measured on a scale of 1-5 after irrigation withdrawal and before flowering (1 = unrolled (turgid), 2 = leaf rim starts to roll, 3 = leaf has a v-shape, 4 = rolled leaf rim covers part of the leaf blade and to 5 = leaf is rolled to the shape of an onion leaf), tassel size measured on a scale of 1-5 (where 1 = few branches, small tassel; 3 = medium size tassel; 5 = many branches, large tassel), grain yield in t ha^{-1} (all harvested ears were hand shelled and grain weight determined at 13% moisture content). Data was tested for normality using $\log_e\surd$ (ASI + 10) and subjected to Analysis of variance (ANOVA) using the general linear model (SAS Version 7). Treatments found to be statistically significant at ($P \leq 0.05$), according to the F-test were subjected to mean separation using the general linear method (SAS version 7).

Table 1: Grouping of Kenyan maize germplasm for drought tolerance evaluation according to Anthesis to Silking Interval (ASI).

Short ASI		**Medium ASI**		**Long ASI**	
Genotype	**ASI days**	**Genotype**	**ASI days**	**Genotype**	**ASI days**
DT/BT/2443.DT	**1**	KTL N 70188-2	**9**	CML-265	**13**
DT/BT/1917.DT	**1**	KTL N 70140-4	**10**	KTL N 10168-2	**13**
GBK-032419	**3**	GBK-045385	**10**	GBK-043227	**14**
DT/BT/1470.DT	**4**	GBK-043731	**10**	KTL N 70133-3	**15**
GBK-032357	**4**	GBK-044611	**10**	KTL N 10162-	**16**
GBK-GBK-027017	**4**	GBK-034711	**11**		
GBK-034659	**5**	GBK-027054	**12**		
CML-492	**5**	Katumani Composite B	**12**		
KTL N 701104	**5**	Makueni Dry Land Composite	**12**		
GBK-044593	**6**				

4 RESULTS AND DISCUSSION

4.1 General observations: Germplasm differed significantly in variables evaluated for drought tolerance (Table 2). Under water stress (WS) conditions, days to tassel anthesis, days to silking, leaf roll intensity and ASI increased while tassel size, number of ears per plant, and grain yield decreased significantly ($P \leq 0.05$). The variability was indicative of the different sources of the germplasm.

4.2 Days to tassel anthesis: In 65% of the germplasm, water stress increased days to tassel anthesis by between 1 and 5 days. In similar findings, Monneveux *et al.* (2005) reported that severe stress prior to flowering induces leaf rolling and reduces stomatal conductance which affects photosynthates partitioning to the male inflorescence (tassel) and hence promotes delayed tassel anthesis. In the composite controls, Katumani Composite B (KCB) and Makueni Dry Land Composite (DLC) water stress increased the days to tassel anthesis by 3 and 4 days, respectively. Water stress did not affect days to tassel anthesis in KTL N 70188-2 and GBK-044593. The control genotypes KCB and DLC exhibited lowest number of days to tassel anthesis (40–45 days) under both moisture regimes. This indicated that these checks had the ability to escape drought. Days to tassel anthesis in the germplasm expected to be drought tolerant based on ASI duration ranged from 57 days in GBK-044593 to 71 days in CML-492, implying that the Composites were superior drought escapers than other the germplasm tested.

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Table 2: Effects of water stress at flowering on the phenotypic characters of Kenyan maize local landraces evaluated at Kibwezi.

. .**Traits**	**Moisture regimes**			
	Water stressed (WS)		**Well watered (WW)**	
	Mean	**Range**	**Mean**	**Range**
Days to anthesis	63.6	43.0-78.0	62.9	40.0-74.0
Days to silking	71.7	53.3-85.0	69.1	48.7-80.7
ASI (days)	7.9	-1.3-12.3	6.3	-1.0-12.3
Leaf rolling (1-5)	3.0	1.7-4.3	1.0	1.0-1.0
Tassel size (1-5)	2.9	1-5	3.3	1-5
Shelling %	68.4	50-83	75	56.7-81.7
Grain yield (t ha^{-1})	1.7	0.4-3.7	3.6	1.8-5.9
Ears/plant	0.9	0.5-1.1	1.1	0.7-1.7
100-seed wt (g)	27.2	15.7-42.1	31.1	19.7-44.6
Plant height (m)	1.7	1.3-2.3	2.1	1.6-2.5

Pr= Probability; CV=coefficient of variation

4.3 Days to silking: Water stress increased the mean days to silking from 69 to 72 days, thus stress caused a delay in silking by 3 days. Richards (2006) similarly found that drought that occurs from mid to late vegetative stage in maize delayed the process of ear silking. Days to silking in the Composite controls ranged from 52 to 54 days in KCB and 49 to 53 in DLC. Inbred lines KTL N 10162-1, KTL N 70133-3, KTL N 10168-2, KTL N 70188-2, KTL N 70140-4, all from Kitale and CML-265 from CIMMYT had the highest number of days to silking (76 to 82 days). Mugo *et al.* (1998) reported that delayed silking was associated with barrenness, and reflected a reduced partitioning of assimilates to the developing ear at flowering, thus reducing yields. Richards (2006) attributes silk delay in the drought-susceptible genotypes to allocation of less assimilates to ear growth during the early stages of development. When flowering occurs under water stress, changes such as accelerated leaf senescence, leaf rolling and reduced stomatal conductance, results in reduced photosynthates flow to the ear.

4.4 Anthesis-silking interval (ASI). Under moisture stress, ASI increased by 2 days. A long ASI duration was an important cause of yield loss and was highly correlated with grain yield, and the number of ears per plant. Anthesis-silking interval is one of the most important traits that can be used to indicate maize genotype's tolerance to stress (Mugo *et al.*, 1998; Richards, 2006). Under such conditions of delayed silking, pollen can arrive after it has desiccated, when silks have withered or senesced or after ovaries have exhausted their starch reserves. Richards (2006) reported that under moisture stress the ASI period was negatively correlated with grain yield. Bolanos and Edmeades (1996) linked a high grain yield under stress to a short ASI. Mugo *et al.* (1998) and Frova *et al.* (1999) similarly found that when drought stress occurs just before or during the flowering period in maize, a delay in silk emergence is observed resulting in an increase in the length of the ASI. Inbred lines DT/BT/1917.DT and DG/BT/2443.DT from KARI-Katumani exhibited lowest ASI duration of -1 day under both moisture regimes i.e. silking before tassel anthesis.

4.5 Leaf rolling. Leaf rolling under water stress was highest in the control genotype DLC, KTL N 70133-3, KTL N10150-1, KTL N 10162-1, GBK-027054 and KTL N 70140-4 with a score of 4 and lowest in DG/BT/2443.DT, DT/BT/1470.DT, CML-492 and CML-265 with a score of 2 (Figure 1). Monneveux *et al.* (2005) associated leaf rolling with a reduction in photosynthesis which is a result of decreased radiation interception. Bolanos and Edmeades (1993) attributed the reduction in photosynthesis to a decrease in radiation interception associated with leaf rolling and reduced leaf expansion. Bolanos and Edmeades (1996) found that leaf rolling is a drought adaptive trait, while Julianno and Kelly (2007) noted ecological, morphological and physiological trade-offs associated with drought adaptation traits.

4.6 Tassel size. Water stress significantly reduced tassel size score from 3.2 to 2.9. The smallest tassel score (1) was recorded in lines KTL N701104, DG/BT/2443.DT and DT/BT/1917.DT. Landraces GBK-043227, GBK-27054 and GBK-034659 exhibited the highest tassel score of 5. Both KCB and DLC controls exhibited medium sized tassel scores of 2.9 and 3.2 respectively. Selection for a small tassel is often associated with improved partitioning to the ear. Lines DG/BT/2443.DT and DT/BT/1917.DT exhibited small tassels, and a low ASI thus better tolerance to drought than those exhibiting large tassels. Monneveux *et al.* (2005) reported that recurrent selection for small tassels resulted in substantial increase in partitioning to early ear growth, increased ear and husk weight, and successful grain set, thus improved grain yield. According to Banziger *et al.* (2000), such genotypes demand less allocation of the little assimilates during times of drought, thus more can be channeled to the developing ear for grain production. Selection for reduced tassel size may also increase canopy photosynthesis through reduced shading. The composite controls flowered and also reached physiological maturity earlier than other germplasm evaluated, indicating that they were superior drought escapers than the landraces.

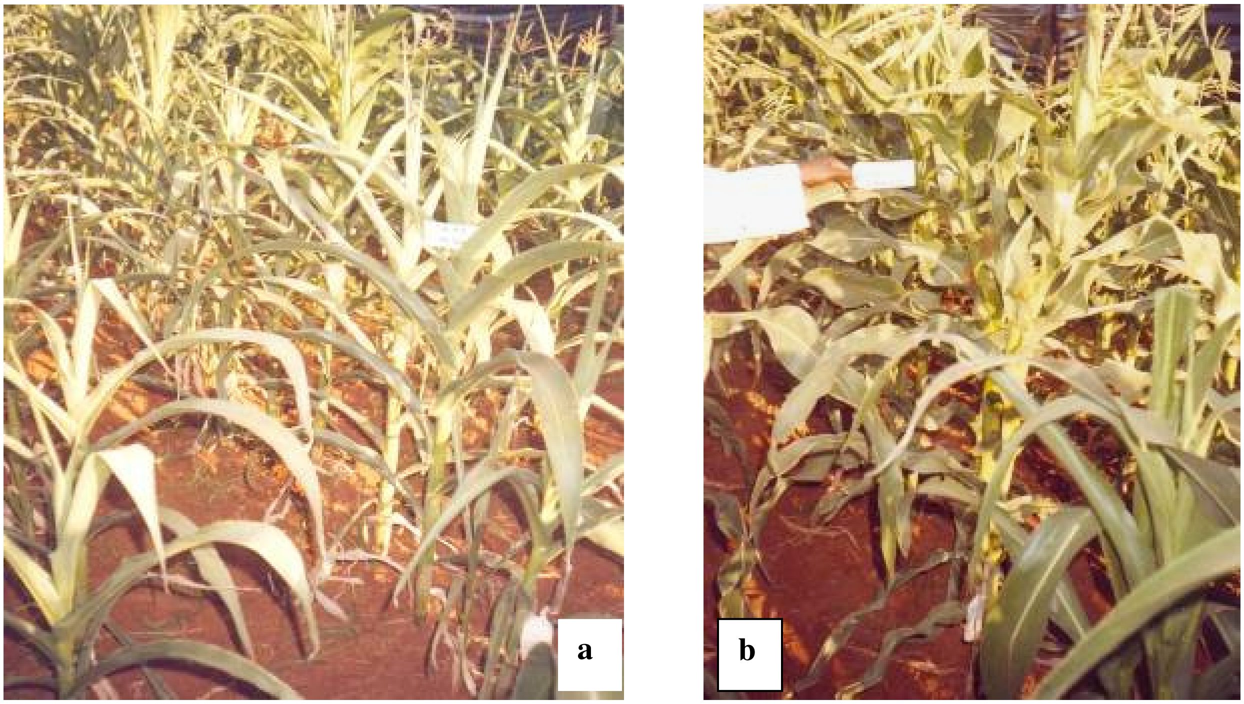

Figure 1: Leaf rolling under moisture stresses for two different genotypes one week after irrigation withdrawal (a) drought susceptible KTL N10150-1 (b) drought tolerant DG/BT/2443.DT

4.7 Number of ears per plant: The number of ears per plant is one of the most important yield components of maize. Water stress significantly reduced mean number of ears per plant from 1.1 to 0.9. Severe stress causes complete abortion of the ear. Under moisture stress, the number of ears per plant ranged from 0.5 in genotype KTL N 70140-4 (80% yield loss) to 1.2 (28% yield loss) in genotype GBK-032419 (Table 3). Under well-watered conditions, it ranged from 0.7 in GBK-027054 to 1.7 in GBK-032419. The mean number of ears per plant under moisture stress was 0.8 and 1.2 under WW conditions. Genotypes GBK-032419, GBK-034659, GBK-032357, KTL N 10168-2, KTL N 701104, CML-492, CML-265, DT/BT/1470.DT and DT/BT/2443.DT had the highest number of ears per plant under moisture stress. Monneveux *et al.* (2005) reported that the number of ears per plant varies with moisture regimes among genotypes. The number of ears per plant under moisture stress for controls KCB and DLC was similar at 0.8; they lost 62 and 68% grain yield, respectively. Mugo *et al.* (1998) noted that grain yield and numbers of ears per plant were inherently smaller in Composites because of the high level of barrenness and floret and kernel abortion. Under moisture stress, a low number of ears per plant could have indicated increased ear barrenness in the genotypes, thus resulting in low grain yields. According to Tollenaar and Wu (1999), grain abortion occurs during the first 2 to 3 weeks after silking and is worsened by stress that reduces canopy photosynthesis and the flux of assimilates to the developing ear. Banziger *et al.* (2000) proposed that on the basis of consideration of heritability and correlation with yield under moisture stress, barrenness should be considered as a useful secondary trait for improving maize yields in drought prone environments. The better performance in some of the genotypes under moisture stress may be a result of the allocation of more assimilates to ear formation at the critical stage. Chapman and Edmeades (1999) and Monneveux *et al.* (2005) attributed grain yield losses to decreasing number of ears per plant under water stress. Bolanos and Edmeades (1996) attributed more than 75% of variation in grain yield under drought to variation in number of ears and kernels per plant. Mugo *et al.* (1998) reported that as stress increases, the dependence of grain yield on ears per plant increased more than on kernels per ear.

4.8 Grain yield: The grain yield of the genotypes varied significantly. Yield reduction under moisture stress ranged from 17% in GBK-034659, a landrace from Taita Taveta to 81% in KTL N 70140-4, a Kitale line. A large fraction of the yield potential was thus not realized in many genotypes under moisture stress (Table 3). Under well watered (WW) conditions, grain yield ranged from 1.8 t ha^{-1} in KTL N 70188-2 to 5.9 t ha^{-1} in GBK-043731, a landrace from Bungoma district. Under moisture stress yield ranged from 0.4 t ha^{-1} in Kitale inbred line KTL N 70140-4 to 3.7 t ha^{-1} in GBK-032419, a landrace from Kilifi. Germplasm GBK-032419, DT/BT/1470.DT and GBK-034659 had the lowest grain yield losses of 28, 22, and 17% under water stress, respectively. Inbred lines DT/BT/2443.DT from Katumani and KTL N 701104 from Kitale were the highest grain yielding lines under WW at 4.6 and 4.1 t ha^{-1}, respectively. Both recorded yield losses of about 50% under water stress. The KCB and DLC controls had 3.7 and 3.6 t ha^{-1}, under WW conditions, and recorded yield losses of 62 and 68%, respectively, under water stress (Table 3). According to Banziger *et al.* (2000), drought tolerant genotypes should perform well under both well watered and water stress conditions. Germplasm DT/BT/2443.DT, DT/BT/1917.DT, GBK-032419, GBK-034659, DT/BT/1470.DT, GBK-032423, GBK-032357, GBK-027017, CML-492, GBK-044593 and KTL N 701104 had lower yield losses under moisture stress than the controls, thus better tolerance to drought than the recommended Composites. They recorded yield losses ranging from 17% in GBK-034659 from Taita Taveta, to 54% in inbred line DT/BT/1917.DT from Katumani. Ouk *et al.* (2006) noted that the practical approach for selection of a drought tolerant parent is to use a measure or an index of the relative grain yield of genotypes under stress to that under well watered conditions as an integrative measure of the complex of traits that provide drought tolerance.

highland germplasm; Kitale inbred KTL N 701104. The most drought susceptible germplasm were KTL N 70140-4, KTL N 10162-1, GBK-043731 and Makueni Dry Land Composite. Since phenotypic characterization of these germplasm has been done, molecular characterization of local landraces identified to be drought tolerant should be carried out for the development of improved maize varieties for dry areas. Local landraces exhibiting superior drought tolerance traits may be evaluated for performance at farmers' level in the marginal areas so that the best landraces could be recommended for use. Some of these landraces were availed to neighboring farmers during this trial.

Table 3: Relative yield loss in the maize germplasm under water stress conditions.

Genotype	**Yield (t/ha) Well watered**	**Yield (t/ha) Water stressed**	**Yield loss (%)**
DT/BT/2443.DT	4.6 (±0.28)	2.2 (±0.95)	52
DT/BT/1917.DT	3.7 (±0.46)	1.7 (±0.43)	54
GBK-032419	5.1 (±2.21)	3.7 (±0.54)	28
DT/BT/1470.DT	3.9 (±2.2)	3.1 (±0.68)	21
GBK-032357	4.7 (±0.69)	1.8 (±0.26)	62
GBK-032423	2.9 (±0.26)	1.6 (±0.49)	45
GBK-027017	3.7 (±0.91)	1.7 (±1.4)	54
GBK-034659	2.9 (±1.15)	2.4 (±0.39)	17
CML-492	2.5 (±0.47)	1.3 (±0.4)	49
KTL N 701104	4.1 (±0.67)	2.0 (±0.55)	51
GBK-044593	3.0 (±0.78)	1.8 (±0.66)	40
KTL N 70188-2	1.8 (±0.75)	1.0 (±0.16)	44
KTL N 70140-4	2.2 (0±.37)	0.4 (±0.36)	81
GBK-045385	4.0 (±0.92)	1.6 (±0.5)	60
GBK-043731	5.9 (±1.7)	1.8 (±0.74)	70
GBK-044611	4.5 (±0.99)	1.0 (±0.53)	77
GBK-034711	3.3 (±0.53)	1.5 (±0.62)	55
GBK-027054	2.0 (±0.64)	1.0 (±0.4)	50
Katumani Composite B	3.7 (±1)	1.4 (±0.43)	62
Dry Land Composite	3.6 (±0.22)	1.1 (±0.97)	68
CML-265	2.2 (±0.29)	1.4 (±0.3)	36
KTL N 10168-2	3.1 (±0.62)	1.3 (±0.7)	54
GBK-043227	4.3 (±0.91)	2.1 (±0.14)	51
KTL N 10150-1	4.2 (±0.9)	1.7 (±0.97)	60
KTL N 70133-3	3.9 (±1)	1.2 (±0.35)	69

Numbers in brackets represent the standard error of the mean

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