**Research Article** 

# Effect of Drought on Seed Germination and Early Seedling of Tomato Genotypes using Polyethylene Glycol 6000

Vincent Ishola Esan<sup>1</sup>, Taiwo Ayanniyin. Ayanbamiji<sup>2</sup>, Janet Omoyemi Adeyemo<sup>2</sup>, Sunday Oluwafemi<sup>1</sup>

<sup>1</sup>Bowen University, Faculty of Agriculture, Department of Environmental Management and Crop Production, Iwo, Osun, Nigeria

<sup>2</sup>Bowen University, Department of Biological Sciences, Faculty of Sciences and Science Education, Bowen University, Iwo, Nigeria

**Abstract:** Tomato is a sensitive crop to a variety of environmental stresses, especially drought. Therefore, the objective of the present work was to screen for drought tolerance at seed and seedling (vegetative) levels for better understanding of drought mechanisms and identification and selection of the most tolerant tomato genotypes. Twenty four (24) tomato genotypes were screened for drought tolerance using 0%, 4% and 14% polyethylene glycol 6000 (PEG 6000). The experiment was laid in complete randomized design with three replication and three treatments. The following parameters: germination percentage, shoot length, root length, shoot and root weight and recovery date were recorded in the course of the experiment. There was no significant difference in germination percentage between control and low PEG. However, significant differences were observed between control and low concentration of PEG when compared to high PEG concentration. Water stress created by PEG 6000 at high concentration (14%) significantly reduced all the parameters measured in all the genotypes. Overall, NGB01357, L00170 and NHGB/09/113 performed better under drought conditions and could be very vital in breeding program.

Keywords: Tomato genotypes, solutions of PEG 6000, germination, water stress

#### Introduction

Tomato (Solanum lycopersicum L.) is an important fruit crop grown worldwide with a total world production of 159 million tons and a value of \$74.1 billion on a cultivated area estimated at 5 million ha (FAO, 2011). It is also one of the most economically important crops and worth a tremendous amount of money because it gives more yields (FAOSTAST, 2013). However, the yield of tomato in West Africa particularly Nigeria is not too promising, especially when compared to that of developed countries. For instance, Nigeria production was estimated at 1,860,600 tonnes in 2010 while the United States of America estimate for the same year was 12,858,700 tonnes (FAO, 2010). Yield per hectare in Nigeria was estimated at 1/7th of that of the U.S.A (FAO, 2010). From nutritional point of view, tomato plays a vital role in providing vitamin C, carotenoids, flavonoids and phenolics for human diet (Horneburg and Myers, 2012). Tomato can be eaten as raw vegetable or can be added to other food items. Many processed products are derived from tomato such as paste, whole peeled tomato, diced products, and various

forms of juice, sauces, and soups have gained significant acceptance. Tomato deserves special attention because of the role it plays in food security in West Africa. However, effort to meet food needs on a sustainable basis for a growing population is seriously reduced by biotic and abiotic stresses. Climate variability and the prevalence of extreme events, in particular drought, are harsh reality for smallholder farmers, which depend exclusively on rainfed agriculture (IPCC, 2007). Climate variability is one of the challenges faced in the world, especially in the developing countries, to maintain the food security.

Drought and flood are the two most severe environmental factors that affect crop production in West Africa. Regrettably, global climate change will increase the occurrence and severity of drought episodes (Tuberosa, 2012). Similarly, Lobell *et al.* (2008) reported that climate change is expected to negatively impact crop production because of variable temperatures and more frequent drought in many parts of the world by the middle of the 21st

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Vincent Ishola Esan (Correspondence)

ishola.vincent@yahoo.com

+234 8065054035

century. This makes development of varieties that can yield well under harsh environments very critical for the prevention of food shortages.

Water is of paramount importance in the production of vegetables and other crops. Worldwide, it is regarded as a restraining factor for production of horticulture and crops (Ghebremariam et al., 2013). Water is crucial for the movement of photoassimilates and nutrients, and very fundamental in all plant physiological processes (Lisar et al., 2012). Therefore, drought is the principal abiotic constraint that causes considerable yield reduction in agricultural production (Robin et al., 2003). It has been estimated that drought accounts for over 70 % of potential agriculture yield losses globally (Boyer 1982). In the same vein, Kulkarni and Deshpande (2008) indicated that crop yields are reduced by 70-80% due to drought specifically during the reproductive stage.

Nowadays, water availability for agriculture is becoming limited alongside with a projected rise in food demand for the growing world population. Therefore, developing novel cultivars with more efficient water-use and greater drought-resistance capacity is the most viable solution to ensure a sustainable agricultural production and alleviate threats to food security. Indeed, the adoption and improvement of crops suited to growth with limited water supply on drought lands is vital to ensuring food security, given the seasonal variability, population growth, and the effects of climate change. In the developing counties, the development and use of crop varieties with high water-use efficiency and high yield is particularly important for areas prone to drought, unreliable rainfall and where irrigation is unavailable or unaffordable for resource-poor farmers. Given that almost 90% of Nigeria's crops are rainfed, the huge seasonal variability associated

Table 1 The name and source of tomato germplasm used

with change in climate such as drought and flood pose a serious threat to farmers. Olaoye (2005) stated that the frequent occurrence of drought occasioned by erratic rainfall distribution and cessation of rains during the growing season is the greatest hindrance to increased production of these food and industrial crops.

Several authors have demonstrated the importance of using inducing chemicals like polyethylene glycol 6000 for the selection of drought tolerance at early germination and seedling stage (Khodarahmpour, 2011; Ghebremariam et al., 2013; Osman Basha et al. 2015; Brdar Jokanović and Zdravković, 2015). It has been reported that using PEG as a selection technique in vitro is the most trustworthy method for screening desirable genotypes and to study further the effects of water scarcity on plant germination indices (Kocheva and Georgiev, 2003; Sakthivelu et al..., 2008).

Tomato is a sensitive horticultural crop to a variety of environmental stresses, especially extreme temperature, drought, salinity and inadequate moisture stresses (Kalloo, 1993). Therefore, the objective of our work was to screen for drought tolerance at germination and seedling levels for better understanding of drought mechanisms, identification and selection of the most tolerant tomato genotypes.

#### MATERIALS AND METHODS Plant Materials

The material used in this study consisted of twenty four (24) tomato genotypes (Table 1). These genotypes were obtained from two different sources: twenty two (22) genotypes from the National Centre for Genetic Resources and Biotechnology (NAGRAB) Ibadan, Nigeria and 2 cultivars were bought from commercial centers of agricultural products (CCAP).

S/No	Name	Source	S/No	Name	Source
1	UC82B	CCAP	13	NGB01250	NACGRAB
2	RomaVF	CCAP	14	NGB1254	NACGRAB
3	NHGB/09/120	NACGRAB	15	L00168	NACGRAB
4	NG/SA/01/10/002	NACGRAB	16	NGB01301	NACGRAB
5	NGB01232	NACGRAB	17	NGB01357	NACGRAB
6	NG/MR/JAN/10/001	NACGRAB	18	NHGB/09/113	NACGRAB
7	NG/MR/MAY/09/005	NACGRAB	19	NG/AA/SEP/09/050	NACGRAB
8	NG/SA/07/10/002	NACGRAB	20	NG/AA/SEP/09/042	NACGRAB
9	NG/MR/MAY/09/006	NACGRAB	21	NG/AA/SEP/09/053	NACGRAB
10	NGB01302	NACGRAB	22	NG/AA/SEP/09/043	NACGRAB
11	NGB01255	NACGRAB	23	L00170	NACGRAB
12	NGB01362	NACGRAB	24	L00169	NACGRAB

# PEG 6000 treatment

Tomato seeds were firstly disinfected with 3% absolute alcohol solution for 10 minutes. They were

then thoroughly washed in 3 changes of distilled water in order to remove the traces of alcohol. In the course of disinfection with alcohol and rinsing with distilled water, tomato seeds were gently shaking for proper sterilization and removal of alcohol traces, respectively. With the aid of forceps twenty five (25) seeds were placed on two layers of Whatman filter papers in 9cm Petri dishes. Three treatments 0%, 4% and 14% were prepared by adding PEG 6000 weighed in scale to distilled water according to the method of Michel and Kaufman (1983) in order to obtain the osmotic potential in PEG. All the petri dishes were kept in the laboratory at a temperature of  $25^{0}C\pm 2$ . Distilled water and prepared PEG 6000 solution were frequently added to the petri dishes as need arose.

#### Measurement

The first reading of seed germination was performed 48 hours after sowing, and then the counting of the germinated seeds was continued every day until the 13<sup>th</sup> day of drought imposition. Shoot length and root length of 10 seedlings from each genotype and each replication were measured on the 13<sup>th</sup> day. Other measurements taken was germination percentage and seed vigor index Germination index (G.I.) was computed by using the following formula:

G.I.= n/d, where, n =number of seedlings emerging on day 'd'

d = day after sowing.

Seed vigor index was calculated by multiplying germination (%) and seedling length (cm). The recovery rate was measured on the 21<sup>st</sup> day. Indeed, non-germinated seeds were removed and placed in new petri dishes with moistened new filter papers and distilled water to observe their recovery rate. It

should be noted that only distilled water was added when required to petri dished for a week.

## Analysis of data

All data recorded were subjected to statistical analysis using "R" software to identify significant difference among the tomato genotypes used under the three treatments of distilled water and PEG 6000. ANOVA was performed for the assessment of the variation at 0.05 level of probability using Newman-Keuls Multiple Comparison-PostHOC test. In addition, Pearson correlation coefficient between traits measured was computed.

## RESULTS

## Germination percentage

Germination percentage is presented in table 2. According to the results of table 1, there is decrease in germination rate as the concentration of PEG 6000 increased. The highest germination percentage was recorded at control while the lowest at 14% PEG 6000 even genotypes NHGB/09/120 and NGB01301 recorded zero germination percentage. There was no significant differences in germination percentage between low concentration (4%) of PEG and control but demonstrated significant difference with high concentration at 14% PEG 6000. Genotypes NGB01357, L00170 and NHGB/09/113 showed the germination percentage under highest high concentration of PEG followed by NG/SA/01/10/002. Apart from NHGB/09/120 and NGB01301 which did not germinate at 14% PEG, NG/MR/JAN/10/001, NG/AA/SEP/09/043, NGB01232 and NGB01250 also recorded very low germination rate 1.33, 1.33, 5.33 and 5.33% respectively.

 Table 2 Germination percentage of tomato genotypes under PEG6000-induced drought stress

Genotypes	Control (0%)	Low (4%)	High (14%)
UC82B	91.67	89.33	14.67ac
RomaVF	90.00	88.00	9.33c
NHGB/09/120	85.33	60.00	0.00b
NG/SA/01/10/002	90.67	87.33	28.00ac
NGB01232	96.67	87.33	5.33c
NG/MR/JAN/10/001	92.67	94.67	1.33c
NG/MR/MAY/09/005	87.33	82.00	22.67ac
NG/SA/07/10/002	94.00	93.67	17.33ac
NG/MR/MAY/09/006	93.00	92.67	20.00ac
NGB01302	89.33	86.00	21.33ac
NGB01255	96.67	95.33	16.00ac
NGB01362	94.00	93.67	13.33ac
NGB01250	92.00	90.33	5.33c
NGB1254	97.33	84.00	16.00ac
L00168	96.00	94.67	13.33c
NGB01301	86.67	84.00	0.00b
NGB01357	92.00	86.67	40.00a
NHGB/09/113	96.33	95.33	33.33a
NG/AA/SEP/09/050	91.33	78.00	10.67c
NG/AA/SEP/09/042	95.33	88.67	10.67cb

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NG/AA/SEP/09/053	90.00	84.67	18.67ac	
NG/AA/SEP/09/043	98.67	77.33	1.33c	
L00170	98.00	88.67	36.00a	
L00169	92.00	88.67	16.00ac	

The same letters in the same column are not significantly different at P<0.05

## **ANOVA** Table of germination

	Df	Sum Sq	Mean Sq	F value	<b>Pr(&gt;F)</b>
Block	2	15.1	7.5	0.8232	0.4411
Variety	23	3221.3	140.1	15.2877	2.2e-16 ***
Treatment	2	7112.2	3556.1	388.1615	2.2e-16 ***
Variety:Treatmt	46	1045.1	22.7	2.4800	2.367e-05 ***
Residuals	142	1300.9	9.2		

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

#### Shoot length and seed vigor

Overall, shoot length decreased as the concentration of PEG increased and the different was significant at high concentration of PEG when compared with control and low PEG concentration (Table 3). No significant difference was observed between control and 4% PEG. The following genotypes NG/AA/SEP/09/053. NG/AA/SEP/09/050. NGB01232. NG/AA/SEP/09/043 and NG/SA/01/10/002 performed better than others at high concentration of PEG 6000 (14%) with the longest shoot length values of 4.88 cm, 4.47 cm, 4.10 cm, 3.20cm and 3.13 cm, respectively. No shoot length was recorded for NHGB/09/120 and NGB01301 at 14% PEG 6000 since there was no seed germination. The shortest shoot length was recorded in NG/MR/JAN/10/001, NGB01357, and NGB01250 with their values of 1cm, 1.23 cm and 1.40 cm, respectively.

Table 3 Mean shoot length in 0%, 4% and 14% PEG 6000 solution

Genotype	0%PEG	4%PEG	14%PEG	Genotype	0%PEG	4%PEG	14%PEG
1	5.31	5.21	1.98	13	4.57	5.86	1.40
2	4.27	4.02	1.60	14	5.77	5.90	2.20
3	5.43	5.32	0.00	15	4.83	4.99	2.73
4	5.36	6.04	3.13	16	5.11	3.74	0.00
5	6.90	5.54	4.10	17	6.25	6.58	1.23
6	3.90	4.34	1.00	18	6.49	6.06	2.20
7	7.84	6.45	3.51	19	4.44	5.29	4.47
8	6.35	5.91	3.10	20	5.11	4.42	2.50
9	6.64	4.82	2.52	21	5.63	5.14	4.88
10	5.61	4.66	2.79	22	5.19	5.07	3.20
11	5.87	5.45	2.63	23	5.35	5.49	2.88
12	5.51	5.45	1.93	24	4.87	4.71	2.89

The highest seed vigor indexes at high concentration of PEG 6000 (Table 4) were recorded in L00170 (103) and NG/AA/SEP/09/053 (91.11) followed by NHGB/09/113 (73.33), NGB01357 (49.2), NG/AA/SEP/09/050 (47.69) and L00169 (46.240. The lowest was observed in NGB01301 (0.00) and NG/AA/SEP/09/043 (4.26).

Genotype	Control	4%	14%	Genotype	Control	4%	14%
1	486.77	465.41	29.05	13	420.44	529.33	7.46
2	384.30	353.76	14.93	14	561.59	495.60	35.20
3	463.34	319.20	0.00	15	463.68	472.40	36.39
4	485.99	527.47	87.64	16	442.88	314.16	0.00
5	667.02	483.81	21.85	17	575.00	570.29	49.20
6	361.41	410.87	1.33	18	625.18	577.70	73.33
7	684.67	528.90	79.57	19	405.51	412.62	47.69
8	596.90	553.59	53.72	20	487.14	391.92	26.68
9	617.52	446.67	50.40	21	506.70	435.20	91.11
10	501.14	400.76	59.51	22	512.10	392.06	4.26
11	567.45	519.55	42.08	23	524.30	486.80	103.68
12	517.94	510.50	25.73	24	448.04	417.64	46.24

Table 4 Mean seed vigor index in 0%, 4% and 14% PEG 6000

#### **Root length**

The mean root length of all tomato genotypes studied is presented in Table 5. Strong and significant differences were not found at 0% and 4% PEG in all the genotypes under the present study. At high concentration, root production was completely inhibited in NHGB/09/120 and NGB01301. In addition, significant root length reduction was observed in other genotypes treated with 14% PEG when compared with control and low concentration. The longest average root lengths were observed in NGB01357, NG/SA/01/10/002, NG/MR/MAY/09/005, NG/MR/JAN/10/001, NGB01250, NG/AA/SEP/09/053, NHGB/09/113, NG/AA/SEP/09/043, NGB1254 and L00170 with their respective values of 5.63 cm, 5.31 cm, 4.90, 4.75 cm, 4.20 cm, 4.19 cm, 4.04 cm, 4.04 cm, 3.82 cm, 3.75 cm. The shortest was found in RomaVF and L00168 with the average values of 1.53 cm and 2.02 cm, respectively.

Table 5 Mean root length in 0%, 4% and 14% PEG 6000 solution

Genotype No	0%	4%	14%	Genotype No	0%	4%	14%
1	4.82	4.89	2.60	13	4.39	5.92	4.75
2	3.60	4.26	1.53	14	6.20	6.69	3.82
3	5.21	5.07	00.00	15	5.01	5.09	2.02
4	8.01	7.16	5.31	16	4.87	4.74	0.00
5	6.76	5.55	3.00	17	8.72	7.57	5.63
6	3.91	4.67	4.20	18	6.03	7.34	4.04
7	8.28	7.42	4.90	19	8.07	7.19	3.08
8	6.88	7.57	3.32	20	5.69	5.18	2.18
9	8.52	7.52	3.62	21	6.60	6.79	4.19
10	6.39	4.78	2.95	22	6.24	6.19	4.00
11	6.43	5.86	3.96	23	5.71	6.78	3.75
12	5.97	5.81	2.52	24	5.48	5.13	2.71

# Fresh weight

Fresh weight of roots and shoots are shown in Figure 1. The fresh weights of RomaVF, NG/MR/MAY/09/005, NG/MR/MAY/09/006, NGB01362 and NGB01232 at 4% PEG were slightly higher than those of the control. The results reveal also that there is no significant difference between fresh weigh of the control and that of low PEG concentration but drastic reduction of fresh weight was found at high concentration (14%) of PEG in all the tomato genotypes. There was no fresh weight recorded in NG/AA/SEP/09/042, NGB01301, NHGB/09/120, NG/AA/SEP/09/050, NG/MR/JAN/10/001, NGB01362, NGB01232, L00168 and NGB01250. The highest fresh weight under 14% PEG 6000 were observed in NG/AA/SEP/09/053, NHGB/09/113, NG/MR/MAY/09/006, L00170, NG/SA/01/10/002, and NGB01357.

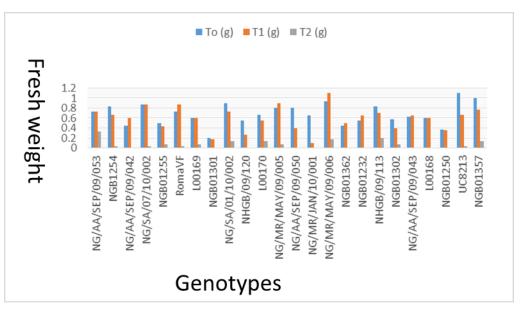


Figure 1 Fresh weight of tomato genotypes under control (T0) 4% PEG (T1) and 14% PEG (T2)

Df	Sum Sq	Mean Sq	F value	<b>Pr(&gt;F)</b>
2	0.4855	0.2427	5.3671	0.005677 **
23	6.2548	0.2719	6.0132	4.44e-12 ***
2	13.1155	6.5578	145.0031	2.2e-16 ***
46	3.5905	0.0781	1.7259	0.008177 **
140	6.3315	0.0452		
	2 23 2 46	2         0.4855           23         6.2548           2         13.1155           46         3.5905	2         0.4855         0.2427           23         6.2548         0.2719           2         13.1155         6.5578           46         3.5905         0.0781	2         0.4855         0.2427         5.3671           23         6.2548         0.2719         6.0132           2         13.1155         6.5578         145.0031           46         3.5905         0.0781         1.7259

#### ANOVA Table of fresh weight

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1

# **Recovery rate**

To find out whether non germinated seed will show recovery and germinate, they were taken from osmotic stressed condition, the non-germinated seeds were transferred to moisten filter papers with distilled water which were placed in plastic petri dishes after 13 days and were observed for additional 7 days. After 2 days, total recovery of germination (100 %) was observed, which showed an indication of drought

# DISCUSSION

Water deficit is one of the most serious environmental factors that affects seed germination, crop growth and development and thus drastically reduces crop productivity and yield around the world (Osman Basha et al., 2015; Lisar et al..., 2012; Mantri et al..., 2012; Van den Berg and Zeng, 2006). Water deficit which lead to drought will definitely be exacerbated by climate change especially in rainfed zones. Therefore, there is urgent need to conduct research through screening for drought tolerance in order to develop new cultivars to be used in drought prone areas. There are several methods of screening crop genotypes but in the present study we chose to conduct the screening exercise at seed germination and seedling level using polyethylene glycols 6000 (PEG 6000) which is regarded as the most trustworthy chemical to induce water stress at the laboratory level. PEG 6000 really demonstrated through this study its ability to discriminate the most tolerant genotypes from the most susceptible ones.

Table 2 shows the germination percentage of each genotype under 3 treatments i.e. 0%, 4% and 14% PEG 6000. NGB01357, L00170 and NHGB/09/113 revealed high percentage germination ranging from 28% to 40% under high concentration (14%) PEG. This indicates that they are the most drought tolerant among the 24 tomato genotypes screened. This could be explained by the fact that these drought tolerant genotypes were able to imbibe water indispensable for germination under osmotic condition created by PEG6000 solution. On the contrary, the most drought susceptible genotypes NHGB/09/120 and NGB01301 were unable to absorb water to trigger their germination and thus failed to record a single seed germination. This is followed by the susceptible genotypes which recorded low germination rate due also to the PEG inhibition. Our results are similar to previous studies on screening different crops for drought tolerance using PEG6000 (Hegarty, 1997; Turk et al..., 2004; Osman Basha et al..., 2015). It should also be noted that 14% PEG had effect on the days of germination because most of the genotypes started germinating on the 4<sup>th</sup> day when compared to those in the control and low PEG concentration (24 hours and 2 days, respectively). Similar results were obtained by Hegarty, (1997) and Turk *et al.*, (2004) who reported that water stress at germination stage delayed germination or impede germination completely.

Decrease in root length was observed with increasing PEG concentration and the reduction was more pronounced in high concentration (Table 5). Similar results were obtained by Jajarmi et al..., (2009); Brdar-Jokanović et al..., (2014b); Toosi et al..., (2014) in their works. In addition to NGB01357, L00170 and NHGB/09/113 with the highest percentage germination under 14% PEG. NG/SA/01/10/002. NG/MR/MAY/09/005. NG/MR/JAN/10/001, NGB01250. NG/AA/SEP/09/053. NG/AA/SEP/09/043 and NGB1254 produced the longest roots which are necessary for water absorption and uptake in deep soil under water stress for better growth, development and high productivity. Our results are consistent with those of Ghebremariam et al..., (2013); and Jokanović and Zdravković (2015). Similarly Kulkarni and Deshpande, (2007) reported that root system is a vital character in drought tolerance.

Low PEG concentration did not affect shoot length of seedlings because their values are similar to that of control even some are slightly higher. Ghebremariam et al... (2013) reported that shoot length was not much affected by the drought situation at low concentration and control. Under high concentration of PEG, the longest shoot of seedlings were observed NG/AA/SEP/09/053. NG/AA/SEP/09/050. in NGB01232 and NG/SA/01/10/002. This illustrates their ability to withstand water stress compared to those that were completely inhibited by drought conditions stimulated by PEG especially at high concentration. Osman Basha et al.., (2015), Toosi et al., (2014), Abdel-Raheem et al., (2007) reported that the increase in PEG concentration decreased shoot length.

For the fresh weight, despite germination was recorded in some genotypes such as NG/AA/SEP/09/042, NGB01301. NG/AA/SEP/09/050, NG/MR/JAN/10/001, NGB01362, NGB01232, L00168 and NGB01250 at 14% PEG, they weighed zero gram i.e. no detectable by the weighing balance (no fresh weight). This could be explained by the fact that PEG did not permit the uptake of water and the partition of stored nutrients in the shoot and roots of the seedlings causing them to be lighter. This is a result of deleterious effect of drought on crop growth and development and biomass.

# CONCLUSION

A breeding program and varietal improvement relies on sufficient genetic variability for target traits. Therefore, the traits studied under PEG stimulated drought conditions allowed us to differentiate between sensitive and tolerant genotypes. Genotypes NGB01357, L00170 and NHGB/09/113 are the most tolerant to drought while NHGB/09/120 and NGB01301 are the most susceptible to drought. PEG 6000 is a reliable chemical to induce drought condition and for rapid screening.

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## **Competing interests**

The authors declare that they have no competing interests

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