**Response to Reviewers comments of the paper entitled:**

**Measuring stolons and rhizomes of turfgrasses using a digital image analysis system**

**Dear Editor:**

**Thank you to you and to the anonymous reviewers for your time and efforts in reviewing our manuscript. Below please find our rebuttal statements (listed in blue) to each of the reviewers’ comments and suggestion for changes.**

**Comments from Peer-Reviewers:**   
  
**Editorial comments:**  
Changes to be made by the Author(s):  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

The text has been checked for spelling and grammar errors.

2. Please revise the following lines to avoid previously published text: 250-254, 262-269, 271-275, 282-284, 286-290, 292-294,

The text has been rewritten.

3. I would recommend not highlighting Step 5 for inclusion in the video as it consists mainly of calculations. Please note that the highlighting is only for the protocol section of the video. There will be an opportunity to discuss the results in the Representative Results section of the video.

The highlight of Step 5 has been removed.

4. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. “This figure has been modified from [citation].”  
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**Reviewer #3:**  
Manuscript Summary:  
The manuscript describe a technically sound piece of scientific research with data that supports the conclusions as well as the efficacy of the protocol. The topic of the present work is within the scope of the Journal. Please consider these minor suggestions that can improve the clarity of the present MS.  
  
Minor Concerns:  
-line 53: "on drought stress tolerance"...not only drought. I ll be more generic. please check here  
<https://www.frontiersin.org/files/Articles/209303/fpls-07-01584-HTML/image_m/fpls-07-01584-t002.jpg>  
<https://doi.org/10.3389/fpls.2016.01584>

The term “drought” has been deleted to be more generic.

-line 54: "Stolons and rhizomes" I suggest to provide a botanical definition to help readers that are not acquainted with turfgrass science

Botanical definition has been added.

-line 150: "using a software application"? are you speaking about a thresholds setting in WinRHIZO, like in the point 3.6?

Threshold settings cannot exclude smaller organs. A correction of length and averaged diameter is needed after the software analysis. The process is described in the Protocol at Step 5.1. To clarify this point a reference to Step 5.1 has been added in line 154.

**Reviewer #4:**  
Manuscript Summary:  
For turfgrass, it is difficult to measure the length and diameter of each stolons or each rhizome using simple rulers and calipers. There are always some errors in traditional measurement methods. Compared with traditional methods of morphology, software-based image analysis system provides an alternative method which seems more efficient and accurate. This article explores the new protocol of morphological measurement which may provide new ideas for the morphological research of turfgrass and other crops. The results of the study indicated a high correlation between new protocol and traditional methods which are of high application value.  
  
Major Concerns:  
Cleaning is an important steps for a successful estimation of length and average diameter of stolons and rhizomes and it is meticulous work requiring special attention. The results show that it took 21 min 24 s for stolons cleaning and 11 min and 12 s for rhizomes cleaning. Measuring length and diameter with a ruler and caliper took an average of 14 min 06 s for stolons and 13 min 35 s for rhizomes. The scan and analysis of the sample using WinRHIZO took an average of 11 min 00 s for stolons and 12 min 04 s for rhizomes. Is software-based image analysis system more quick and convenient in the measurement of stolons and rhizomes in practical applications? It seems that the new method does not have much advantage because of cleaning time.  
The cleaning is necessary for both measurements: software-based and ruler/caliper-based. The time necessary to have scanned and hand-measured data is almost the same when limited number of stolons/rhizomes is involved. Differences between the two methods concern measurements, the software-based method allows to analyse a limited or large number of organs taking the same time, the limiting factor is the scan surface size. On the contrary, the time necessary to measure organs by hand increases with the number of organs. The discussion has been improved about this issue in Lines 399-405.  
  
**Reviewer #5:**  
I have reviewed the revised manuscript entitled "Measuring stolons and rhizomes of turfgrasses using a digital image analysis system". This manuscript presents the WinRhizo software application for measuring some morphometric parameters of stolons and rhizomes of bermudagrass. Thus it is scope of JoVE journal. However, I do not recommend this manuscript for publication because of two reasons.  
The first problem is autoplagiarism. Authors republished significant part of data (Figure 1-4) from their article in Agronomy Journal (doi:10.2134/agronj2017.03.0187).  
The second shortcoming is a lack of research problem. According to the producer, Regent Instruments Inc, the algorithms in WinRhizo allow to measure not only root parameters. WinRhizo is optimized for root measurements but it can be used to analyze other objects e.g. leaves. Thus, there is no novelty in WinRhizo application in stolon measurements.  
Moreover, description and comparison of different cultivars of bermudagrass is also not a research problem. The differences in plant morphology between cultivars are usually very obvious. In this research not any explanation (or consequences) for these differences are presented.

As suggested, this comment has not been answered.  
  
  
**Reviewer #6:**  
Manuscript Summary:  
An interesting manuscript that describes protocols for measuring stolon and rhizome length in Bermuda grasses using digital image software WinRHIZO. Correlations obtained for hand v digital analysis of stolon/rhizome length and stolon/rhizome diameter were >0.98 and 0.8, respectively.  
  
Major Concerns:  
Rhizomes can easily penetrate down to 15 cms of the soil profile rather than 5 cm used in this study.

In previous version of the manuscript we presented data from perennial ryegrass samples. Vegetative organs in creeping perennial ryegrasses are stolons which grow at the soil surface or just below ground and samples were collected with a core sampler 8 cm diameter and 5 cm depth. Depth of samples presented in this paper was a misprint from the previous version. The depth has been corrected at line 108 and 304. The correct samples depth was 15 cm, based on our experience rhizomes of bermudagrass turf do not grow below this depth (Rimi, F. 2012. Performance of warm season turfgrasses as affected by various management practices in a transition zone environment. PhD thesis. University of Padova).

Also, this manuscript is data poor, do the authors have additional data that can be used?

The Result section has been improved (Figure 6).

Did the correlations vary for different genotypes?

As we described in the Results section, correlation was very good for length measures. We tested different cultivars and demonstrated that the precision of length measurements is not affected by the cultivar. Cultivars, species, genotype, could affect the correlation between measured and scanned diameter if they differ for morphological traits involving nodes. As reported in the text (lines 386-389, “the average diameter estimated by means of this protocol cannot be compared to the internode diameter measured with a caliper. The digital image analysis includes nodes and internodes in the calculation of average diameter, that is the ratio between total projection surface and total length.” Furthermore, “Stolon diameter is typically used to describe the diameter of stolon internodes and is a common parameter used for botanical description. For this reason, Pornaro et al. pointed out that average diameter estimated through WinRHIZO system and the manually measured internode diameter describe two different morphology aspects.”